Principles of Cultivar Development

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Iowa State University is located on the ancestral lands and territory of the Baxoje (bah-kho-dzhe), or Ioway Nation. The United States obtained the land from the Meskwaki and Sauk nations in the Treaty of 1842. We wish to recognize our obligations to this land and to the people who took care of it, as well as to the 17,000 Native people who live in Iowa today.

Contents

Preface

The goal of *Principles of Cultivar Development*, and that of the preceding *Plant Breeding Methods*, is to help you learn how to be a successful manager of a cultivar development program. In *Principles of Cultivar Development,* you will apply the principles learned in *Plant Breeding Methods* to design a breeding program that makes effective use of available resources and alternative breeding strategies to develop clonal, pure-line, hybrid, and synthetic cultivars.

The topics for the chapters in *Principles of Cultivar Development* are Maximizing genetic gain, Development of clonal cultivars, Development of pure-line cultivars, Heterosis and hybrid vigor, Development of hybrid cultivars, Development of synthetic cultivars, Multilines and seed blends, and Release and distribution of cultivars.

In this book, we have designated additional reading that you should complete to fully understand the concepts in each chapter. The information in the text has been augmented with additional material that includes examples of how breeders have implemented the concepts in their breeding programs, videos, and pictures to help you visualize the methods.

Maximizing genetic gain I

Walter R. Fehr and Walter P. Suza

Readings:

- Chapters 6 and 7 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)
- Chapter 17. 18 and 19 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr

Introduction

A successful program of cultivar development is based on the effective utilization of available resources for **selection** of superior individuals for traits of importance. The amount of available resources, including facilities, time, and money, and the traits of importance in a new cultivar differ for every breeding program. As a result, no two breeding programs are designed the same. This is evident by comparing the breeding strategies used by different persons to develop cultivars of a plant species that are published in the *Journal of Plant Registrations* and *Horticultural Science*.

This section is intended to help you understand the variables that need to be considered in designing an effective breeding program for selection of traits that are quantitatively inherited. Our recommended reading for this section from *Principles of Cultivar Development* chapter 17, contains a formula that can be used to compare the amount of genetic improvement per year from different breeding strategies.

$$G_c = h^2 D$$

Each of the components in the formula can be influenced by the choices breeders make in carrying out their cultivar development programs. Chapters 6, 7, 18, and 19 in *Principles of Cultivar Development* are important for understanding how each of the components can be manipulated to maximize genetic improvement.

The formula for genetic gain per year in Chapter 17 of *Principles of Cultivar Development* is based on the variance component method of calculating heritability. The components used in the formula are derived from analyses of variance, examples of which are provided in tables 17-6 and 17-7 of the chapter (pp. 227–228), reproduced below.

Table 17-6, Analysis of Variance for Seed Weight (g/100 Seeds) of 60 F45 Lines of Soybeans Tested in Two Replications at Two Environments, with Three Individual Plants Evaluated From All Plots. Source: Frank, 1980

Source	Degrees of Freedom	Mean Squares	Expected Mean Squares*
Total	719	7.68	
Exvironments (E)	1	281.28	
Replications/E (R/E)	2	27.45	
Lines (L)	59	59.33 M_1	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2 + nrt\sigma_g^2$
E × L	59	4.00 M_2	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2$
(R/E) × L	118	3.25 M_3	$\sigma_w^2 + n\sigma^2$
Plants/plots	480	2.20 M_4	σ_w^2

*n = plants per plot = 3; r = replications = 2; t = environments = 2.

$\sigma_w^2 = M_4 = 2.20$

$\sigma^2 = (M_3 - M_4)/n = [(\sigma_w^2 + n\sigma^2) - \sigma_w^2] = (3.25 - 2.20)/3 = 0/35.$

$\sigma_e^2 = M_3/n = (\sigma_w^2 + n\sigma^2)/n = 3.25/3 = 1.08$

$\sigma_{ge}^2 = (M_2 - M_3)/nrt = [(\sigma_w^2 + n\sigma^2 + nrt_{ge}^2) - (\sigma_w^2 + n\sigma^2)]/nr = (4.00 = 3.25)/6 = 0.125$

$\sigma_g^2 = (M_1 - M_2)/nrt = [(\sigma_w^2 + n\sigma^2 + nrt_{ge}^2) - (\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2) + nrt\sigma_g^2]/nrt = (59.33 = 4.00)/12 = 4.61.$

$\sigma_{ph}^2 = M_1/nrt = (\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2 + nrt\sigma_g^2)/nrt = (\sigma_w^2/nrt) + (\sigma^2/rt) + \sigma_g^2 = 59.33/(2 \times 3 \times 2) = 4.94.$

Source: Frank, 1980

Table 17-7, Analysis of Variance for Seed Weight (g/100 Seeds) of Random Sample of 20 of 60 F4:5 Lines Analyzed in Table 17-6 (Three Plants Were Evaluated for All Plots of 20 Lines)

Source	Degrees of Freedom	Mean Squares	Expected Mean Squares*
Total	239	5.58	
Exvironments (E)	1	50.47	
Replications/E (R/E)	2	7.81	
Lines (L)	19	40.08 M_1	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2 + nrt\sigma_g^2$
E × L	19	3.53 M_2	$\sigma_w^2 + n\sigma^2 + nr\sigma_{ge}^2$
(R/E) × L	38	2.29 M_3	$\sigma_w^2 + n\sigma^2$
Plants/plots	160	2.16 M_4	σ_w^2

Heritability is a numerical measure of the reliability of selection for a quantitative trait. The primary value of calculating heritabilities is to compare the expected gain from different strategies of selection, as will be done in Applied Learning Activities 1 and 3. In Applied Learning 1, you will compare the relative effectiveness of selection among individual seeds versus among individual plants for oleic acid. In Applied Learning Activity 3, you will compare the expected genetic gain per year for different methods of selection.

Although heritability is a useful tool for comparing breeding strategies, breeders commonly decide on the

reliability of selection without calculating a heritability value. The data for Applied Learning Activity 1 will be used to illustrate this point. In that assignment, you are asked to compare the realized heritability of data obtained from individual seeds versus individual plants derived from the seed. The one with the greatest heritability would be considered the most reliable for selection. However, a breeder may choose to practice selection even if the heritability is low in order to discard seeds or plants that have very little chance of being effective.

With the data for Applied Learning Activity 1, the breeder would decide on the oleic content of the $F_{2:3}$ lines that would be acceptable. Assume that 50% is an acceptable value. The next step would be to look at the oleic acid value of the seed and plant for each of the lines with >50% oleic acid. In this example, the seed with the lowest value for lines with >50% oleic acid was 40.86% and the plant with the lowest value was 42.42%. The breeder may decide that it would be reasonable in the future to discard all seeds and plants with less than a certain percentage of oleic acid so that time and resources would not be spent evaluating individuals that have little promise of being useful.

Applied Learning Activity 1

You have been hired by a company to breed cultivars for increased oleic acid content, which is a quantitative trait. You want to know if it is practical to select for the trait among single F_2 seeds or among individual F_2 plants. To help you decide, you conduct an experiment that will make it possible to compute realized heritabilities in a single-cross population formed by crossing a mid-oleic parent with about 60% oleic acid to a normal parent with about 25% oleic acid.

The hybrid F_1 plants from the population were harvested in bulk, a total of 50 F_2 seeds were analyzed for oleic acid content non-destructively, and each analyzed seed was identified and planted the following season. The identified F_2 plants were harvested individually and the oleic acid content of each was determined by gas chromatography with a bulk sample of five F_3 seed. The progeny of each F_2 plant was grown as a $F_{2:3}$ line in a multiple location trial, and the oleic acid content was determined for each $F_{2:3}$ line from each location. The oleic acid content for each F_2 seed, F_2 plant, and $F_{2:3}$ line are provided. For this assignment, you will need to submit your answers for each of the following 10 parts.

1. Mark with a highlighter the five F_2 seeds with the highest oleic acid content. Calculate the mean oleic acid content of the selected F_2 seeds. Calculate the mean of all the F_2 seeds. Subtract the mean of all the F_2 seeds from the mean of the five selected seeds. The remainder will be the denominator for calculating the realized heritability for individual seeds. Show your calculation.

2. Mark with the same color highlighter as used in step 1 the oleic acid content of the $F_{2:3}$ lines that trace to the selected F_2 seeds. Calculate the mean oleic acid content of the five $F_{2:3}$ lines you marked with a highlighter and the mean of all the $F_{2:3}$ lines. Subtract the mean of all the $F_{2:3}$ lines from the mean of the five $F_{2:3}$ lines that you had selected as F_2 seeds. The remainder will be numerator for calculating the realized heritability for individual seeds. Show your calculation.

3. Divide the remainder in step 2 by the remainder in step 1. The quotient is the realized heritability for individual seeds. Show your calculation.

4. With a different colored highlighter, mark the five F_2 plants with the highest oleic content. Calculate the mean oleic acid content of the selected F_2 plants. Calculate the mean of all the F_2 plants. Subtract the mean of all the F_2 plants from the mean of the five selected plants. The remainder will be the denominator for calculating the realized heritability for individual plants. Show your calculation.

5. Mark with the same color highlighter used in step 4 the oleic acid content of the $F_{2:3}$ lines that trace to the selected F_2 plants. Calculate the mean oleic acid content of the five $F_{2:3}$ lines you marked with a highlighter. You calculated the mean of all the $F_{2:3}$ lines in step 2. Subtract the mean of all the $F_{2:3}$ lines from the mean of the five $F_{2:3}$ lines that you had selected as F_2 plants. The remainder will be numerator for calculating the realized heritability for individual plants. Show your calculation.

6. Divide the remainder in step 5 by the remainder in step 4. The quotient is the realized heritability for individual plants. Show your calculation.

7. Why would expect the heritability to be greater for selection among individual plants than individual seeds?

8. What are reasons to explain why the heritabilities were less than 100%?

9. What percentage of oleic acid would you be comfortable to use for discarding seeds? Why?

10. What percentage of oleic acid would you be comfortable to use for discarding plants? Why?

- Download the data from table 1: **Chapter 1_ALA_Data [XLS]**

Review the full data table below:

Table 1.

Entry	F_2 seed	F_2 plant	$F_{2:3}$ line
1	43.43	37.51	46.21
2	50.15	31.24	45.78
3	37.73	31.41	29.43
4	32.27	31.83	30.80
5	44.59	35.31	37.33
6	53.97	58.25	51.84
7	42.44	30.44	30.43
8	52.00	67.06	49.43
9	50.25	59.42	54.37
10	46.94	55.74	41.47
11	48.05	38.78	37.34
12	42.39	36.32	26.91
13	50.66	35.74	38.83
14	46.61	42.42	50.85
15	58.64	55.12	50.89
16	65.28	62.70	49.72
17	58.19	48.81	37.62
18	57.23	47.80	51.38
19	34.60	54.55	42.51
20	41.68	43.43	53.83
21	26.51	33.98	33.18
22	41.64	61.38	52.72
23	27.43	35.88	41.20
24	32.06	41.34	32.95
25	50.58	59.24	49.45
26	27.64	35.13	31.96
27	27.83	33.26	36.43
28	32.48	43.80	39.85
29	31.23	36.82	38.16
30	40.86	48.31	50.09
31	33.49	26.58	36.97
32	33.12	32.47	43.60
33	32.55	37.61	40.14
34	66.30	45.14	49.19
35	50.50	48.60	50.76
36	37.30	42.27	49.79

Entry	F_2 seed	F_2 plant	$F_{2:3}$ line
37	26.33	30.92	41.35
38	31.05	27.53	34.22
39	34.33	39.33	43.02
40	29.18	24.41	29.09
41	62.95	49.13	53.41
42	55.05	38.71	41.77
43	44.33	27.14	31.01
44	53.27	27.58	26.05
45	51.27	48.12	40.62
46	46.97	39.87	33.90
47	54.32	38.17	35.92
48	53.28	44.64	52.40
49	36.65	58.35	38.88
50	43.05	43.98	45.69

References

Fehr, W. R. (ed). 1987. Principles of Cultivar Development. Vol 1. Theory and Technique. McGraw-Hill, Inc., New York.

Maximizing genetic gain II

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 18 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)

Introduction

The emphasis of the lesson is on the role of **genotype x environment interaction** on the effectiveness of selection for quantitative traits. The importance of this interaction is highly dependent on the trait under selection. For example, the relative difference among genotypes for the number of days from planting to maturity is much more consistent among environments than the differences among genotypes for seed or forage yield. As a result, the genotype x environment component in the genetic gain equation discussed in the previous chapter is smaller for days to maturity than for yield. Therefore, a breeder can use fewer environments to obtain reliable values for the maturity of a genotype than are necessary to determine the genetic potential of a genotype for yield.

From the farmers' perspective, performance data in one season is used to select cultivars for planting the next season. When a trait has a low genotype x environment interaction, the relative differences among cultivars in one season will be similar in the coming season. For example, a cultivar that matures 10 days earlier than another in one season would be expected to mature earlier in the coming season, although not necessarily by 10 days. For yield, one cultivar that is higher yielding than a second cultivar in one season may be lower yielding than the second cultivar in the next season.

In conducting research to determine the importance of genotype x environment interaction in a breeding program, a significant interaction may be found in an analysis of **variance**. Whether or not the interaction is of importance to the breeder depends on which of the types (illustrated in chapter 18 of *Principles of Cultivar Development*) are involved. The most difficult interaction to deal with is one that results when the best genotypes in one environment perform less well than others in another environment. When this type of interaction occurs, the breeder generally uses less stringent selection in one environment when deciding which genotypes to advance to the next season of testing.

The breeder ultimately relies on test results from multiple locations and years for making the decision on whether a genotype merits release as a clonal or pure-line cultivar or as a component of synthetic or hybrid cultivar. The purpose of Applied Learning Activity 2 is to illustrate the challenge that genotype x environment interaction presents to the breeder for making selections.

Applied Learning Activity 2

The following data are from the M.S. thesis of Raechel Baumgartner at Iowa State University in which she evaluated the total tocopherol (Vitamin E) content in the oil of soybean lines. There were 20 lines with mid-oleate content of about 50% grown in a randomized complete-block design with two replications at each of three Iowa locations. The goal of the breeding program is to develop cultivars with high Vitamin E content.

The genotype x environment interaction was significant for population 2, but not for populations 1. Provide answers for each of the following questions and an explanation for each answer.

1. What are two primary causes or types of genotype x environment interaction? How does each type affected selection of lines by a breeder?

2. Which of the two types of interactions are responsible for the significant genotype x environment interaction for mid-oleate lines in population 2? It is possible to both types of interactions to be involved in a significant genotype x environment interaction.

3. Based on the phenotypic correlations among the mean values for the individual lines, do you think that genotype x environment interaction would likely make it more or less difficult for a breeder to select the best lines in population1 than in population 2, even though the analyses of variance for population 1indicated that the interaction was not statistically significant?

4. Which lines, if any, would you be comfortable selecting in each population based on one environment of data? Give the designation of the lines for each population that you would be comfortable selecting. Keep in mind that any genotype you advance for additional testing will utilize your financial resources that always are limiting in a breeding program.

5. To minimize the impact of genotype x environment interaction on genetic gain in a breeding program, would it be more important to emphasize the number of environments used to evaluate lines or the number of replications at each environment? Use the genetic gain equation to defend your answer.

6. How would the heritability of a trait relate to the amount of testing required to determine the genetic potential of an individual for that traits? Compare a trait of your choice that has a relatively low heritability and another that has a relatively high heritability. Use a real example from the literature or your own experience, not a hypothetical example.

Table 1. Phenotypic correlation coefficients for total tocopherol content among locations for mid-oleate lines from three populations.

Population	Location	Carlisle	Rippey
1	**Ames**	0.20ns†	0.41ns
1	**Carlisle**		0.25ns
2	**Ames**	0.86**	0.85**
2	**Carlisle**		0.94**

- * significant at the 0.05 probability level
- ** significant at the 0.01 probability level
- † ns = not significant at the 0.05 probability level

Table 2. Means and ranks for total tocopherol content of 20 mid-oleate lines for Population 1 at three Iowa locations.

Entry	Mean (Ames), mg kg^{-1}	Rank (Ames)	Mean (Carlisle), mg kg^{-1}	Rank (Carlisle)	Mean (Rippey), mg kg^{-1}	Rank (Rippey)	Overall Mean	Rank
418001	1646	20	1799	14	1820	12	1755	17
418002	1786	16	1924	8	1648	17	1786	16
418003	1824	13	1474	20	1111	20	1469	20
418004	2078	1	2010	3	1737	14	1942	8
418005	1677	19	1945	6	2019	4	1880	9
418007	2041	4	1952	4	1993	8	1995	3
418008	1843	12	1877	10	1789	13	1836	12
418010	1925	11	1805	12	1633	18	1787	15
418011	1949	9	1950	5	1980	9	1960	6
418012	1798	14	1788	15	1927	10	1837	11
418014	1948	10	1926	7	1652	16	1842	10
418016	1681	18	1640	17	1702	15	1674	18
418017	1949	8	2012	2	2044	2	2002	2
418018	2035	5	1820	11	2008	6	1954	7
418019	1978	7	1485	19	1993	7	1819	14
418020	1727	17	1726	16	1489	19	1647	19
418022	2046	3	1533	18	2324	1	1968	5
418024	1995	6	1909	9	2032	3	1979	4
418026	1788	15	1803	13	1865	11	1819	13
418027	2053	2	2017	1	2008	5	2026	1

Table 2 Appendix

	Mean, Ames	Mean, Carlisle	Mean (Rippey)	Overall Mean
LSD 0.05	220	777	642	340
LSD 0.01	295	1040	859	451

Table 3. Means and ranks for total tocopherol content of 20 mid-oleate lines for Population 2 at three Iowa locations.

Entry	Mean (Ames), mg kg^{-1}	Rank (Ames)	Mean (Carlisle), mg kg^{-1}	Rank (Carlisle)	Mean (Rippey), mg kg^{-1}	Rank (Rippey)	Overall Mean	Rank
419002	1885	10	1800	10	1992	7	1892	9
419006	1730	19	1621	18	1734	17	1695	19
419007	1917	8	1816	9	2002	6	1912	8
419008	1809	16	1631	17	1689	19	1710	18
419009	1987	4	1898	3	2108	1	1998	3
419010	2006	3	1886	5	2071	4	1988	4
419011	2030	2	1957	1	2075	3	2021	2
419012	1953	7	1865	7	2017	5	1945	5
419013	1862	13	1597	19	1729	18	1729	17
419014	1844	14	1737	13	1857	13	1813	13
419015	1711	20	1580	20	1665	20	1652	20
419017	1954	6	1891	4	1972	9	1939	7
419018	1875	11	1671	16	1795	16	1780	15
419020	2135	1	1924	2	2091	2	2050	1
419021	1785	18	1701	14	1842	15	1776	16
419022	1901	9	1745	11	1943	10	1863	10
419023	1798	17	1686	15	1896	12	1793	14
419025	1979	5	1876	6	1977	8	1944	6
419026	1838	15	1841	8	1908	11	1862	11
419027	1863	12	1738	12	1851	14	1817	12

Table 3. Appendix

	Mean, Ames	Mean, Carlisle	Mean (Rippey)	Overall Mean
LSD 0.05	116	88	69	73
LSD 0.01	155	118	93	98

References

Fehr, W. R. (ed). 1987. Principles of Cultivar Development. Vol 1. Theory and Technique. McGraw-Hill, Inc., New York.

Maximizing genetic gain III

Walter R. Fehr and Walter P. Suza

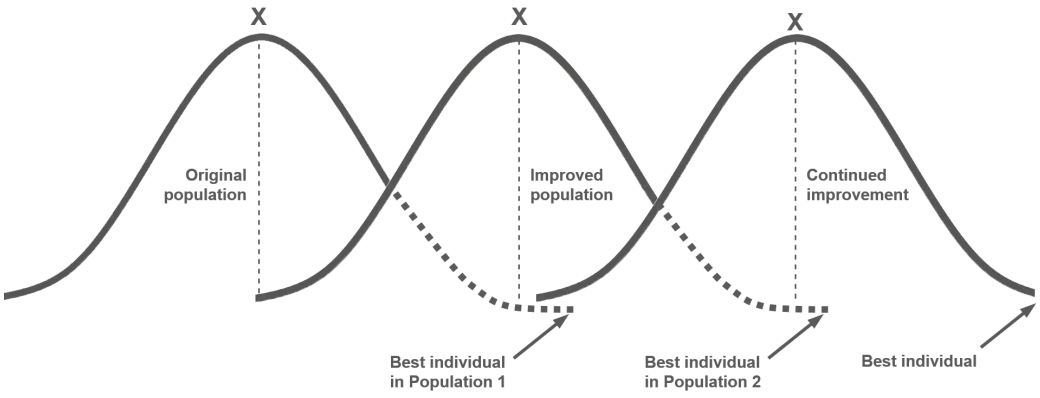
Readings:

- Chapters 6 and 7 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)
- Chapter 15 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr
- Chapters 17, 18, and 19 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr

Introduction

The Applied Learning Activity for this lesson is intended to give you experience in applying the principles that are discussed in the reading assignment. The answers you obtain should be useful for understanding the options a breeder has for maximizing the amount of genetic gain per year with the resources available.

The genetic gain equation was developed as a method of comparing methods of recurrent selection. As indicated in chapter 15 of *Principles of Cultivar Development*, **classical recurrent selection** means that once a segregating population is formed, only superior progeny from the population are crossed together to begin the next cycle of selection.



Classical recurrent selection, figure by Abbey Elder

Although this is done, many breeders would say that any breeding program is essentially recurrent selection, even though superior progeny from different populations and sources are used to form their new segregating populations for selection each year. With regard to the valuable principles that can be learned from the genetic gain equation, it does not matter if a breeder is conducting classical recurrent selection with closed populations or a more traditional breeding program in which the superior progeny identified each year from multiple populations are used to form new populations.

The values for each of the components in the genetic gain equation vary with every breeding population, quantitative trait, and plant species. You will learn more about how to derive the components in your statistics courses and in the course on population and quantitative genetics for breeding. The critical thing to learn in this lesson is how the breeder can influence the magnitude of each component in the equation to maximize genetic gain per year.

In the section of *Plant Breeding Methods* dealing with recurrent selection, it was pointed out that the methods of recurrent selection of practical use for a plant species depend on the type of cultivar under development. Applied Learning Activity 3 assumes that all of the methods are practical to use for a species, so that you can see how the estimated genetic gain can vary.

The practical experience of the breeder plays a major role in making decisions about the methods of recurrent selection that are the more effective. For example, the use of **selfed** families is possible for population improvement in crops for which hybrids are used commercially, such as maize. However, research has demonstrated that the amount of genetic gain per year for the yield of hybrids in maize is less when selfed families are used for evaluation than when individuals are evaluated as **half-sib** families for their combining ability. This is consistent with how breeders conduct a breeding program for developing improved inbreds, as will be discussed in the section on **hybrid cultivars**. Breeders rely on tests of combining ability for yield, instead of on the yield of an inbred line per se.

One of the most important principles that the genetic gain formula teaches is the importance of completing a cycle of selection as rapidly as possible, regardless whether you are doing classical recurrent selection or more conventional breeding. It is the genetic gain per year and not the genetic gain per cycle that is critical. That is why modern plant breeding programs make such extensive use of off-season nurseries to reduce the number of years it takes from the time a cross is made to form a segregating population until superior individuals from the population have been selected for use as parents to form new populations. One plant breeding myth is that it does not matter how long it takes to develop a cultivar, as long as you have new ones to release on a consistent basis. This myth fails to take into account the importance of genetic gain per year. Over a 24-year period, a breeder who takes only four years from crossing until superior progeny are ready to use as parents will make substantially more genetic gain than a breeder who takes eight years to complete a cycle.

When you prepare for the mid-term and final exam of this course, be sure you can write out the entire formula for genetic gain per year and can explain how you as a breeder can influence the magnitude of each of the components in the formula.

Applied Learning Activity 3

You have the following estimates available for making the calculations required in this problem. The relative magnitude of the additive genetic variance and the additive x environment interaction versus the dominance genetic variance and dominance x environment interaction varies among traits. For this problem, assume that the genetic variance is divided equally between the additive and dominance variance ie. 82 for the additive and 82 for the dominance variance. For the genotypic x environment interaction, assume that the additive x environment and the dominance x environment both equal 48.

$$\sigma_u^2 = 1280 \qquad \sigma^2 = 184 \qquad \sigma_{ge}^2 = 96 \qquad \sigma_g^2 = 164$$

Your research manager wants you to evaluate alternative methods of recurrent selection that could be used to improve a population for an important quantitative character. For genotypic evaluation, assume that lines are evaluated in two replications with 30 plants per plot at each of five locations. The selection intensity is 10% for all methods.

1. Compute genetic gain per cycle for the following methods. Put your answers in the table below. The gain should be expressed to the nearest 0.1.

    a. Recurrent phenotypic selection – selection before flowering with gridding

    b. Recurrent phenotypic selection – selection after flowering without gridding

    c. Half-sib selection, population as tester, recombine with half-sib seed

    d. Half-sib selection, inbred tester, recombine with selfed seed. Assume $\sigma_g^2 = 1/4\sigma_A^2$

    e. $S_{0:1}$ line evaluation – 1 intercrossing generation between cycles

    f. $S_{2:4}$ line evaluation – 2 intercrossing generations between cycles

2. Compute gain per year for each of the six methods in part A under the following circumstances. The gain should be expressed to the nearest 0.1. To determine the number of years required to complete a cycle of selection for each of the methods and seasons, it is best to count the number of years it takes to go from the season of testing individuals from the cycle 0 population until you begin testing individuals from the cycle 1 population.

    For example, assume you are evaluating half-sib selection with the population as the tester and have only season per year available. If a test of the half-sib families from cycle 0 was done in Ames during 2012, you would select your best individuals based on data collected by the end of the year, cross the best individuals to form new half-sib families of cycle 1in 2013, and select the best individuals from

cycle 1 based on data for half-sib data collected by the end of 2014, which would be two years per cycle. On the other hand, if you had two similar seasons each year, you could test the half-sib families for cycle 0 in Ames during 2012, use the selected individuals to form new half-sib families of cycle 1 during a winter season in Hawaii, and test the new half-sib families during 2013, which would be one year per cycle. It helps in determining the number of years per cycle to make up locations of your choice for each of the scenarios below. Keep in the mind that in some of the scenarios, the number of years per cycle can be part of a year, such as 1.5.

a. One season per year for testing, crossing, or selfing

b. Two similar seasons per year. Testing, crossing, or selfing can be done in any season

c. Two non-similar seasons per year. Testing is possible in only one season, but crossing and selfing can be done in both seasons.

d. Three seasons per year. Testing, crossing, or selfing are possible in one season, followed by two consecutive seasons in which only crossing and selfing are possible.

Expected gain from selection

Method	Gain per cycle	Gain per year (1 season)	Gain per year (2 similar seasons)	Gain per year (2 non-similar seasons)	Gain per year (3 seasons)
a.					
b.					
c.					
d.					
e.					
f.					

3. Which of the methods would you choose for the four circumstances below? Consider amount of gain per year and cost of the gain for the six methods.
   a. One season per year

   b. Two similar seasons per year

   c. Two non-similar seasons per year

   d. Three seasons

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Clonal cultivars

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 33 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)
- Chapter 3 [PDF], *Hybridization of Crop Plants*, by Walter R. Fehr and Henry H. Hadley (Eds.) (Access the full book)

There are three basic steps in the development of a cultivar that is planted commercially by the use of clones.

Step 1. *Develop a segregating population.*

The cultivars or experimental clones used as parents are highly **heterozygous** and **homogeneous**. When two parents are crossed, it does not matter how many plants of each parent are used because all the plants of a parent are genetically identical. When two parents are crossed, every locus of a parent that is heterozygous will segregate, which means that every gamete produced by a parent is genetically different. Consequently, when the gametes of the two parents unite, every hybrid seed is genetically different. Although the hybrid seed can be referred to as the F_1, it represents the segregating population that the breeder generally will use for selection. Therefore, the breeder attempts to obtain as many hybrid seed of a cross as are desired for sampling the genetic variation of the segregating population. Accidental self-pollination generally is not a concern because the resulting plants will have inbreeding depression and will be discarded due to inferior performance.

Plant species that reproduce by apomixis represent a unique class of clonal cultivars because they are propagated commercially by seed. The two types of apomixis are facultative and obligate. Facultative apomicts, like Kentucky bluegrass, seed is produced asexually and sexually. The asexual seed is genetically identical to the mother plant on which it is borne. The sexual seed is genetically heterogeneous because it results from the union of gametes from heterozygous plants. Obligate apomicts, like buffelgrass, rarely produce any seed sexually. Additional information about the two types can be found in chapter 2 of *Principles of Cultivar Development* and pages 41–62 of *Hybridization of Crop Plants*.

The sexual reproduction of facultative or obligate apomicts is necessary to form a segregating population. Seed harvested from the female parent will include both asexual and hybrid seed. When the seed is planted, the individuals that are identical phenotypically to the female parent are considered to have arisen through apomixis and are discarded. Those that are different from the female parent are assumed to have arisen through sexual reproduction and are used for selection.

The difficulty in crossing parents of obligate apomicts is the limited amount of sexual reproduction. Methods of overcoming this problem are discussed in pages 57–60 of *Hybridization of Crop Plants*.

Step 2. *Select superior plants (clones).*

Every hybrid plant obtained from the cross of two clonal cultivars is a potential new cultivar. The first phase of selection is to identify the individual hybrid plants that have the characteristics desired in a new cultivar. The next phase is to clonally propagate the selected individuals for more extensive evaluations that occur over multiple locations and years. An example of this evaluation process is outlined for potato on pages 386–387 of *Principles of Cultivar Development, Vol. 2*.

Step 3. *Production and commercial distribution of seedstock*

This will be covered in more detail below. An example of the process of developing a clonal cultivar is provided for sugarcane in the following presentation by Sheilah Oltmans-Deardoff:

Step 4: *Preparation of seedstock for commercial planting*

The focus here is on the production and commercial distribution of seedstock. The term “seed” refers to vegetative propagules, such as tubers and rhizomes, or true seed of species that reproduce by apomixis, such as Kentucky bluegrass or buffelgrass.

One of the challenges in the multiplication of clonally propagated cultivars is the potential for the seedstock to carry diseases, particularly viruses. Diseases can be transmitted by contact with an infected clone or the tools used for propagation. To prevent this from happening, various methods of managing seedstock have been adopted.

Example 1: Potato

The testing of potato seedstock (tubers) to detect the presence or absence of transmissible viruses, also known as virus-indexing, is a requirement in North America. An excellent summary of the propagation procedures used to generate disease-free potato seed-stock was developed by staff at Oregon State University, archived here: Propagation Procedures, Foundation Potato Seed Program [PDF].

One explanation for the absence of viruses in meristem tissue is their lack of vascular bundle cells, which eliminates the mode of transport of the virus and restricts their spread. Another explanation is that cells in meristem tissues divide more rapidly than the rate of virus replication. Thus, new meristems tissue escapes virus infection.

Other methods of eliminating viruses in potato tubers involve heat treatment or a combination of both meristem culture and heat treatment. Rare mutations can occur during the tissue culture process. To ensure that plants produced by tissue culture are of high quality and uniformity as their parents, molecular markers such as SSR can be used for cultivar identification.

Figure 1. Virus-indexing of potato. Meristem tissue is isolated and small explants (circled) are collected and placed in sterile test-tubes containing the appropriate growth medium. The tissue culture-derived plantlets are later transferred to the greenhouse and monitored for disease symptoms (left lower panel). The tubers produced in the green house (right lower panel) will be grown in the field for tuber increase. Photos courtesy of Shui-zhang Fei, Iowa State University.Virus-indexing involves collection of small sections of tuber sprouts, or shoot apical meristem, and regenerating plants in sterile medium supplemented with necessary nutrients (Figure 1). Stringent measures are taken to ensure meristem cultured plants are disease-free until their subsequent transfer to the greenhouses.

Example 2: Sweet potato

The production of virus-free sweet potato involves the use of meristem culture to produce plants that are later tested for the presence of viruses by grafting them onto a virus-sensitive (indicator) relative of sweet potato known scientifically as *Ipomea setosa*. After grafting, the indicator plant that is used as the rootstock is monitored for disease symptoms. If no symptoms are observed on the indicator plant, the meristem-cultured plants used as the scions are considered virus-free.

The procedure is described in greater detail by Xu et al (2024).

Example 3: Banana

All the banana diseases are thought to spread through the exchange of vegetative stock (suckers) among farmers. Virus-indexing for banana involves the collection of suckers from the field and growing them in disease monitoring facilities. The plants are screened for diseases and, if negative, are used for tissue culture production of disease-free stocks. Genetic tests using DNA markers may be applied to establish that the

plants produced through tissue culture are the same genetically as their parent. Virus- and disease-free stocks are subsequently distributed to farmers.

For more information about the production of disease-free banana seedstock, see this report on Managing Banana and Citrus Diseases [PDF].

Example 4: Kentucky bluegrass

'Awesome', JPR 5:5-10 (2011)

Awesome was derived from a cross between 'Limousine' as the female parent and Midnight' as the male parent. Limousine seed was sown in flats and allowed to germinate in the greenhouse. The seedlings from the greenhouse were transferred to a field nursery. Plants that looked different from Limousine were flagged and their seed was harvested individually for subsequent evaluation. Awesome is different from Limousine with respect to size, shape and color of its seedheads. The average level of apomixis in Awesome is about 95%. Awesome is protected under the United States Plant Variety Protection (PVP) Act. A description of seed production of Kentucky bluegrass is provided in this presentation: Kentucky_bluegrass_seed_prod [PPT]

Applied Learning Activity 4

Answer the following questions for each of the four cultivars listed below:

- Paspalum – 'Aloha', JPR 5:22-26 (2011)
- Redbud – 'Ruby Falls', Hort. Sci. 45:146-147 (2010)
- Strawberry – 'Valley Sunset', Hort. Sci. 45:663-665 (2010)
- Sweet potato – 'Liberty', Hort. Sci. 46:125-129 (2011)

1. What were the characteristics that the breeder wanted in the new cultivar when the breeding program was designed?

2. What were the parents used to form the segregating population?

3. Outline season-by-season how the selection process was carried out from the time individual plants from the segregating population were grown until the final selection of the cultivar was made. When indicated by the author, describe the number of individuals from the population that were evaluated each season, whether individual plants or plots with multiple plants were used for evaluation, and the number of locations and replications used for testing.

4. What were the characteristics of the individual that led to its release as a clonal cultivar?

Applied Learning Activity 5

For the following four cultivars, provide the following information:

- Paspalum – 'Aloha', JPR 5:22-26 (2011)
- Redbud – 'Ruby Falls', Hort. Sci. 45:146-147 (2010)
- Strawberry – 'Valley Sunset', Hort. Sci. 45:663-665 (2010)
- Sweet potato – 'Liberty', Hort. Sci. 46:125-129 (2011)

1. What part of the plant is used for commercial propagation?

2. What is the method used to multiple and prepare the seedstock for distribution?

3. Who distributed the seedstock commercially?

4. What legal protection was sought for the cultivar, if any.

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Pure-Line Cultivars

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 8 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)
- Chapters 22 to 27 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr
- Chapter 31 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr

There are four steps in the development of pure-line cultivars.

Step 1: *Develop a segregating population.*

The first step in developing a pure-line cultivar is to develop a **segregating population**. The most common type of population is obtained by crossing two elite parents. A three-parent cross or **backcross population** is used when it is unlikely that a two-parent cross will have an adequate frequency of segregates with one or more of the traits desired in the new cultivar. The use of more complex populations generally is limited to situations in which the breeder chooses to do recurrent selection for multiple cycles for improvement of a particular trait. For example, recurrent selection for improved resistance to iron chlorosis on high pH soils was carried in soybean. The initial cycle 0 population was obtained by intermating 10 elite lines and 10 plant introductions with the highest level of resistance to iron chlorosis available. Recurrent selection by the use of $S_{0:1}$ lines was successful in developing genotypes that had an exceptionally high level of resistance to iron chlorosis.

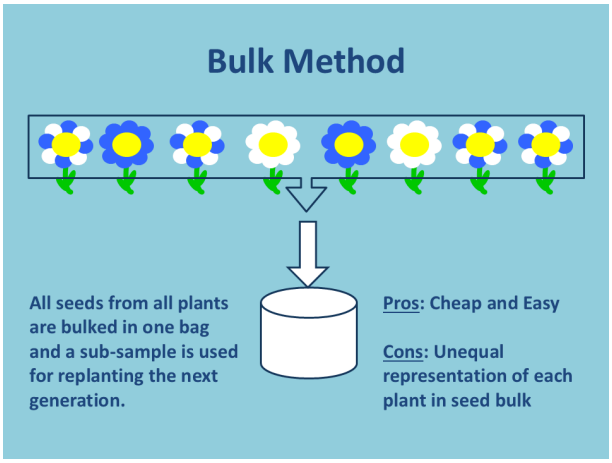
In making crosses between pure-line parents, it generally is assumed that the parents are homozygous and homogeneous for quantitative traits. Consequently, the breeder will only use as many plants of each parent for crossing as is necessary to obtain the desired number of hybrid seed. The number of hybrid seed will depend on the type of population that is developed. For a two-parent cross, all the hybrid seeds are considered genetically identical; therefore, the breeder will obtain enough to generate the size of the F_2 population that is desired. For a three-parent or backcross population, the hybrid plants from the initial two-parent cross is crossed to a third parent or to the genotype used as the recurrent parent. In the second cross, the breeder will try to obtain as many hybrid seed as possible to sample the segregating gametes from the two-parent F_1 individuals.

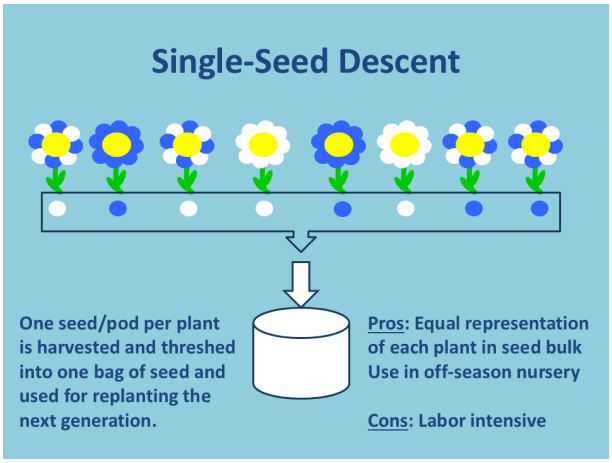
Step 2: *Develop pure lines.*

You need to review and fully understand the alternative methods of developing pure lines that were discussed in the Inbreeding chapter in *Plant Breeding Methods*. As indicated in those lessons, the method used by the breeder will be determined by the resources available and their personal experience

The generation when lines are derived for testing as potential cultivars is an important decision that must be made by the breeder. The advantage of deriving lines in an early generation, such as the F_2 or F_3, is that it takes fewer years to develop a cultivar. The disadvantage is that frequency of lines with the desired level of homogeneity will be less in early generations than for more advanced generations. The generation of deriving lines often is influenced by the breeding schedule that commonly includes the use of the local environment and off-season nurseries. For example, if crosses are made in the local environment, the breeder may be able to grow the F_1 and F_2 generations in an off-season nursery, followed by selection among F_3 plants in the local environment for subsequent testing as F_3-derived lines.

An example of the process of developing a pure-line cultivar is provided for soybean in the following images:





The following slides demonstrate the development of lines by the soybean breeding program of Iowa State University:

ISU_Soybean_Breeding_Slides [PPT]

In this example, the crosses are made at Ames during the summer, the F_1 seeds are planted near Santa Isabel, Puerto Rico, during the middle of October, the F_1 plants are harvested in January, the F_2 seeds are planted in early February, the F_3 seeds are harvested in May by the multiple-seed procedure of single-seed descent, the F_3 seeds are planted at Ames in May, and individual F_3 plants are harvested individually in the fall to form F_3-derived lines for subsequent evaluation.

Step 3: *Testing of pure lines as potential cultivars*

The third step in the development of pure-line cultivars is the testing of lines until one is identified that merits release as a cultivar. Initial evaluation of pure lines for yield and other traits generally is done in a limited number of replications and environments with a relatively small plot size. The objective of the

initial evaluation is to discard lines that the breeder feels are too inferior to warrant further evaluation. The number of lines evaluated and the percentage of lines selected depends on the quality of the parents used to form the population, the number of traits that a line must have to be selected, the frequency of lines with an adequate level of homogeneity, the stringency of the breeder in making selections, and the number of lines that can be tested the next season. Practical experience with a crop is needed to adequately manage these variables effectively.

For most self-pollinated species, the seed that is used for planting a field test is the self-pollinated seed harvested from the previous season of testing. This assumes that the equipment used for harvest is adequately self-cleaning to minimize seed mixtures from one plot to the next. Specialized harvest equipment is available for this purpose.

Step 4: *Seed purification and multiplication for a new cultivar*

An important decision that the breeder must make is when to begin seed purification and increase of lines that have the potential to become new cultivars. The least expensive strategy is to delay the process until the testing is completed and the new cultivar has been chosen for release. If this strategy is used, it delays the time when seed of the new cultivar will be available to the producer. Most breeders prefer to have seed available to producer as soon as possible, which means they carry out seed purification and multiplication concurrently with field testing. The disadvantage of this procedure is that funds will be spent on seed multiplication of lines that ultimately are not released.

The two methods used to produce breeder seed of a pure-line cultivar are **mass selection** and **progeny testing**.

Mass selection

Seed of a line inspected for uniformity is planted in an increase. The seed commonly is obtained from the previous year of yield testing and may or may not be inspected for uniformity before it is planted. The plants in the increase are inspected for uniformity, off-types are removed, and the remaining plants are harvested in bulk to obtain breeder seed. The advantages of mass selection are that it is requires only one season and a minimum amount of labor and expense. The disadvantage is that the genetic and phenotypic uniformity of the breeder seed generally is less than when progeny testing is used. A description of the use of mass selection for breeder seed production was provided for the spring barley cultivar Transit (JPR 5:270-272).

Progeny testing

When progeny testing is used for breeder seed production, individual plants are selected from a line, a progeny row is grown of each, and the rows are evaluated for uniformity and similarity to each other for phenotypic traits. The selected progeny rows may be harvested in bulk or each may be harvested separately and inspected for seed traits before bulking. If the breeder chooses, seed from progeny rows may be harvested separately and each may be grown separately a second season to further examine them for uniformity before they are bulked. The advantage of progeny testing is greater genetic and phenotypic uniformity of the breeder seed than is only mass selection is used. This can be important when a cultivar

must meet standards for genetic purity for the seed certification. The disadvantages of progeny testing are that it takes at least two seasons and requires more labor and expense than mass selection. A description of the use of progeny testing for breeder seed production was provided for the lentil cultivar Essex (JPR 5:19-21).

Applied Learning Activity 6

The following four pure-line cultivars are described in *Journal of Plant Registrations*.

- 'Transit' spring barley – JPR 5:270-272 (2011)
- 'CL151' rice – JPR 5:177-180 (2011)
- 'Snowglenn' winter durum – JPR 5:81-86 (2011)
- 'Bailey' peanut – JPR 5: 27-29 (2011)

For each cultivar, answer the following questions.

1. What type of cross (single cross, three-way cross, or other) was used to obtain the segregating population in which selection was practiced? Why do you think the breeder chose this type of cross?

2. What type of parents (experimental line, cultivar, or other) was used to develop the population?

3. In what generation was the cultivar derived?

4. Outline season-by-season the method used to derive the line. If the details are not clear, use your best judgment in describing what likely was done. End your answer when the line is ready for testing to determine its merits as a new cultivar.

Applied Learning Activity 7

Answer the following questions for each of the four pure-line cultivars below.

- 'Dan' winter hulless barley – JPR 5: 1-4 (2011)
- 'Avalanche' navy bean – JPR 5:170-176 (2011)
- 'Barlow' hard red spring wheat – JPR 5: 62-67 (2011)
- ' Merl' soft red winter wheat – JPR 5: 68-74

1. Describe season-by-season the number of locations and replications used for testing from the time the cultivar was derived until it was selected for release.

2. Describe the method the breeders used to prepare breeder seed.

3. What type of intellectual property protection, if any, was used for the cultivar?

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Heterosis

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 9 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)
- Chapter 35 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr
- Chapter 4, *Breeding Vegetable Crops*, by Mark J. Bassett (Ed.) (Access online via Internet Archive)

Hybrid cultivars are preferred over pure-line or synthetic cultivars for plant species in which the yield of grain or forage is the primary trait of economic importance. One reason for the interest in hybrid cultivars is that they often yield more than pure-line or synthetic cultivars. The second reason is that it is not practical for a farmer to save seed of a hybrid cultivar to plant the following season because the harvested seed will segregate like an F_2 population. As a result, seed companies are assured that the farmer will have to buy new hybrid seed every planting season. In contrast, seed of a pure-line or synthetic cultivar that is harvested one season can be planted the following season.

There are four requirements that must be met to produce a commercially viable hybrid cultivar of a plant species.

Requirement 1: *Heterosis*

Heterosis, commonly referred to as hybrid vigor, can be expressed in two ways. Mid-parent heterosis is when the performance of the hybrid exceeds the mean performance of its parents. Although this type of heterosis commonly is measured in scientific studies, it is not of commercial interest because one of parents may perform better than the hybrid. High-parent heterosis is when the hybrid performs better than either parent. A plant species must exhibit sufficient high-parent heterosis to be commercially viable.

The genetic and molecular basis of heterosis remain unresolved. There are some scientists that believe it may be a combination of all of the possible causes (See chapter 9 in *Principles of Cultivar Development*). Their hypothesis is that heterosis for the alleles at some loci may be caused by dominance, for others it may be overdominance, and for others it may involve epistatic interactions.

Heterosis is measured by evaluating the hybrid performance from mating two or more parents. The ability of a parent to mate with others to produce a superior hybrid is referred to as its combining ability. Specific combining ability refers to the ability of a parent to combine specifically with another parent. General combining ability is the average ability of one parent to combine with a group of other parents. These terms will be discussed further in the lessons dealing with hybrid and synthetic cultivars.

For breeding hybrid cultivars, parent germplasm for some species is divided into heterotic groups. Hybrid cultivars generally have greater performance when the inbred or clonal lines developed from one heterotic group are mated to genotypes from another heterotic group than when genotypes from the same heterotic group are mated to each other. Maize is an example of a species in which heterotic groups are important for maximizing the performance of hybrid cultivars. One heterotic group is referred to as the Iowa Stiff Stalk Synthetic, which was developed by corn breeders of the USDA-ARS and Iowa State University. The other heterotic groups are referred to as non-Stiff Stalk. They include the maize populations Lancaster and Reid Yellow Dent. Breeders find that the best hybrid performance generally is obtained by crossing inbreds from the Stiff Stalk Synthetic with those from one of the other heterotic groups.

Heterotic groups sometimes arise as a result of the mechanism used to produce commercial hybrid seed. For example, use of cytoplasmic-genetic male sterility (CMS) involves the use of female inbreds (A-lines) whose genes in the cytoplasm and nucleus result in male sterility. The male inbreds (R-lines) have restorer genes that override the genes in the cytoplasm and result in a male fertile hybrid. Breeders keep separate the germplasm used for developing the two types of lines, which ultimately leads to unique heterotic groups. The same principle applies to the use of self-incompatibility for producing hybrid cultivars of some crops. Separate germplasm pools with different genes for self-incompatibility are maintained, which leads to unique heterotic groups.

Requirement 2: *Elimination of fertile pollen from the female parent*

Preparation of the female parent for hybridization involves the elimination of fertile pollen to prevent self-pollination. Table 8.1 lists methods commonly used in hybrid vegetable seed production.

Table 8.1. Commonly used methods/mechanisms to eliminate pollen from the female parent and introduce pollen of the male parent for commercial production of hybrid seed of vegetable crops (Adapted from Kumar and Singh, 2004)

Method/mechanism used	Examples of crops
Hand emasculation plus hand pollination	Tomato, eggplant, sweet pepper, okra, hot pepper
Pinching of staminate flowers plus hand pollination	Curcubits (bitter gourd, bottlegourd)
Male sterility plus hand pollination	Tomato, hot pepper, sweet pepper
Male sterility plus natural pollination	Onion, cabbage, cauliflower, carrot, radish, muskmelon, hot pepper
Self-incompatibility plus natural pollination	Most of the cole vegetables like broccoli, cabbage
Gynoecism plus natural pollination	Cucumber, muskmelon
Pinching of staminate flowers* plus natural pollination	Curcubits (bitter gourd, summer squash)
Plant growth regulator of staminate flowers* plus natural pollination	Summer squash, winter squash

*Genotypes with increased proportions of pistillate flowers are desirable for hybrid development.

Manual emasculation

In some species, the elimination of fertile pollen from the female parent of a hybrid is accomplished by hand emasculation of perfect flowers or manual removal of staminate flowers from flowers from monoecious plants (Kumar and Singh, 2004). Tomato will be used to illustrate the principles associated with the use of manual emasculation in a species with perfect flowers. An excellent description of the manual process used to obtain commercial hybrid seed of tomato was developed by Opeña, Chen, Kalb, & Hanson (2001) [PDF].

There are some important requirements for the practical use of manual emasculation for species with perfect flowers. (1) The cost of hand labor must be low enough so that the cost of the hybrid seed is acceptable to the consumer. Consequently, the production of hybrid seed by manual labor generally is done in countries where labor is less expensive than in the United States. (2) The number of hybrid seed from a single pollination should be high. In tomato, the number of hybrid seeds from a single pollination (fruit) is about 200. (3) The value of a hybrid plant must be sufficiently greater for the end user than that of a pure-line cultivar.

Maize is an example of a monoecious species for which manual removal of the staminate flowers (detasseling) for commercial hybrid seed production is commonly practiced. Manual emasculation also can be practical in plant species with monoecious flowers (Kumar and Singh, 2004). A description of the detasseling process can be found in this detasseling video (https://youtu.be/W3Ar4sdL-JI).

Genetic (nuclear) male sterility

Major nuclear genes have been found in many plant species that cause the pollen to be inviable, the most common of which are recessive. The primary use of the genes for hybrid seed production has been in vegetable crops in which they provide an alternative to hand emasculation, including tomato, pepper, and okra. The problem with use of genetic male sterility is that the female parent of the hybrid segregates for male-fertile and male-sterile plants. Plants of the female parent must be inspected individually and the male-fertile ones removed before pollination, which is labor intensive and expensive process. These costs are only acceptable for producing seed of species for which hybrid plants are valuable enough to justify the expense.

Pepper will be used to illustrate the principles involved with the use of genetic male sterility (Hundal and Dhall, 2004). Genetic male sterility in pepper, as in many other species, is controlled by the recessive allele *ms*. One method that can be used to develop the inbred line used as the female parent for hybrid seed production begins with selecting male fertile F_2 plants (*MsMs* or *Msms*) in a population segregating for the *ms* allele, as described in Chapter 16 of the text. The *Msms* plants are identified by progeny testing the $F_{2:3}$ lines and selecting those segregating for male-fertile and male-sterile plants. Within the segregating rows, multiple individual male-fertile F_3 plants are harvested and progeny tested as $F_{3:4}$ lines to identify those that are segregating. Male-fertile F_4 plants are harvested within the segregating line and progeny tested as in the previous generations. The process is continued until the lines are considered adequately homozygous. If a line is selected for use in a hybrid, seed is harvested from its male-sterile plants that have been pollinated by male-fertile plants in the same line. The seed from the male-sterile plant will be male-sterile (msms) and male-fertile (*Msms*). In subsequent generations of seed production, the only male-fertile plants present will

be *Msms* and the resulting seed used to plant the female parent for hybrid seed production will segregate in a ratio of 50% male sterile and 50% male fertile. The male-fertile plants are removed by hand before pollination. The remaining male-sterile plants are pollinated by the male-fertile male parent (*MsMs*). The male parent can be developed by use of conventional inbreeding methods.

An alternative method for developing the female inbred is to first identify an elite male-fertile (*MsMs*) line, followed by backcrossing the *ms* allele into it. This would eliminate the need for identifying *Msms* individuals within a line during inbreeding, which would simplify the inbreeding process. However, it would take additional time to carry out the backcrossing program after the elite line was identified.

The ideal situation for eliminating the male-fertile plants in the female parent would be to have a gene tightly linked to the *Ms* allele that produces a plant phenotype that can be readily observed before flowering. Plants with that phenotype would be discarded as male-fertile individuals. Such linkages have been proposed for many years, but none have been used in hybrid seed production .

Gynoecious inbreds

Gynoecious inbreds that have only pistillate flowers are used as the female parent for hybrid seed production in some monoecious and dioecious species. This method of eliminating male pollen from the female parent will be illustrated with asparagus, a dioecious species, that has genes on X and Y chromosomes that control sex expression and with cucumber, a monoecious species, for which the genes controlling sex expression are not associated with X and Y chromosomes.

Asparagus

Asparagus is a dioecious species with X and Y chromosomes that determine whether a plant has female or male flowers (Ellison, 1986; Anido and Cointry, 2008). Female plants have XX chromosomes, males have XY, and supermales have YY. Most commercial hybrids are produced by mating a gynoecious female parent (XX) to an androecious supermale inbred (YY) to obtain a androecious male hybrid (XY). Male hybrids yield more and live longer than females. The primary commercial method of vegetatively propagating the male hybrid is with plants derived from meristem tissue culture. For home use, male hybrids can be propagated by pieces of the rhizome (crown).

According to Dr. Chin, the asparagus breeder at Rutgers University (http://aesop.rutgers.edu/~asparagus/research/PROGRAM.HTML), the primary methods currently used for obtaining inbred lines is by self-pollination of andromonecious (XY) (Figure 1) and anther culture.

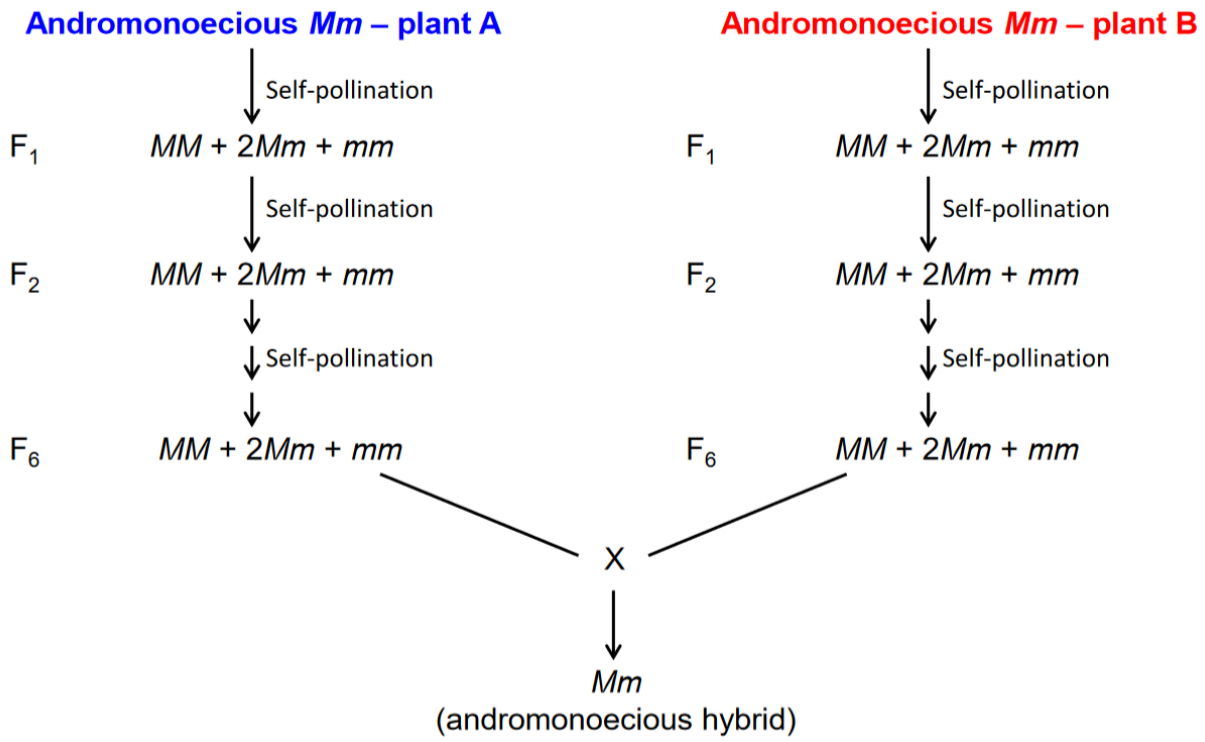

Figure 1. Scheme for inbreeding andromonoecious asparagus to produce uniform male hybrids. Sex determination in asparagus is determined by a single dominant gene, designated M. All plants with the Mm genotype are male, also called andromonoecious. Those with the MM genotype are referred to as supermale. Female plants (gynoecious) have the mm genotype. Adapted from Ellison, (1986)

An andromonoecious plant produces enough hermaphroditic (perfect flowers) for self-pollination. After the breeder considers a line to be adequately homozygous, the gynoecious females (XX) or the supermales (YY) in the selfed progeny are selected. According to Dr. Chin, the best hybrids commonly are obtained by crossing a female from one location of origin to a supermale from another location of origin.

For anther culture, anthers from male (XY) plants in the population are used to generate homozygous female (XX) and homozygous supermales (YY). Although the frequency of such individuals is low, fewer years are required to generate potential inbreds than by self-pollination.

Cucumber

There are three major genes that control the flower type and distribution for cucumber. The gene symbols used for the three genes differ among authors, such as that used by Lower and Edwards (1986) in *Breeding Vegetable Crops*. In this lesson, the symbols *M*,*m*; *F*,*f*; and *A*,*a* will be used as reported by Call and Wehner (2010) and Staub et al. (2008).

The three flowering types of practical importance for producing hybrids are gynoecious (*MMFF*), monoecious (*MMff*), hermaphroditic (*mmFF*), and andromonoecious (*mmff*). The *A*,*a* alleles do not influence these four flowering types. The androecious type has the genotype *ffaa* and is not influenced by the *M*,*a* allele. The andromonoecious and androecious types generally are not involved in breeding or production of commercial hybrids.

Several types of hybrids are used commercially in cucumber. Gynoecious hybrids are preferred for commercial-scale production because they flower earlier than monecious hybrids and all of the fruit can be harvested mechanically at one time, instead of multiple harvests with monoecious hybrids. Monoecious hybrids are used for home gardens. A description of seed production of these hybrid types can be found at Call and Wehner (2010). Information on field production of cucumber can be found at Valenzuela, Hamasaki, and Fukuda (n.d.).

Gynoecious inbred (*MMFF*) x Gynoecious inbred (*MMFF*) = Gynoecious hybrid (*MMFF*). For the production of hybrid seed, silver nitrate or silver thiosulfate are sprayed on the male parent to inhibit formation of the plant hormone ethylene, which stimulates the production of staminate flowers. About 45% of cucumber acreage in the USA is of this hybrid type (Staub, personal communication, 2010). For field production, about 15% of monoecious hybrid seeds that serve as pollinators are blended with the gynoecious hybrid seed before planting (Staub et al., 2008). Gynoecious x gynoecious hybrids commonly are part of the system used for parthenocarpic production of seedless cucumbers in the greenhouse. Parthenocarpic cucumbers are formed without pollination. The parthenocarpic trait is under separate genetic control than the *M* and *F* genes that control the gynoecious trait (De Ponti, 1976). Therefore, the breeding of gynoecious lines for parthenocarpic production is more complex than breeding gynoecious lines for use in hybrids whose fruit is produced through pollination in the field.

The selfing of gynoecious plants for inbreeding is accomplished by treating them with silver nitrate or silver thiosulphate to stimulate the production of staminate flowers. The methods used for generation advancement are discussed further in *Plant Breeding Methods*. Use of the methods in cucumber is described by Staub et al. (2008).

Gynoecious inbred (*MMFF*) x Monoecious hybrid (*MMff*) = Gynoecious hybrid (*MMFf*). This hybrid type is common used for large-scale commercial in the field. About 15% of monoecious hybrid seeds that serve as pollinators are blended with the gynoecious hybrid seed before planting.

The selfing of monoecious plants for inbred line development can be readily accomplished because there are pistillate and staminate flowers on the same plant.

Gynoecious inbred (*MMFF*) x Hermaphroditic inbred (*mmFF*) = Gynoecious hybrid (*MmFF*). About 15% of monoecious hybrid seeds that serve as pollinators are blended with the gynoecious hybrid seed before planting. This hybrid type is not used in the United States because the shape of the fruit produced by the hybrid is not usually acceptable.

Monoecious inbred (*MMff*) x monoecious (*MMff*) inbred = Monoecious hybrid (*MMff*). For production of hybrid seed, the growth regulator ethephon (brand name Ethrel) is applied to the female parent to increase the production of ethylene, which results in the formation of pistillate instead of staminate flowers. This hybrid type is desirable for home use because flowering and fruit production extend over a longer period of time than for gynoecious hybrids. It also is used in countries such as Mexico for long-season production of cucumber (Todd Wehner, North Carolina State Univ., personal communication, 2010)

Hermaphroditic inbred (*mmFF*) x hermaphroditic (*mmFF*) inbred = Hermaphroditic hybrid (*mmFF*). This

type of hybrid is not grown in the United States because the unattractive spherical fruit with big blossom-end scars is not acceptable to consumers (Jack Staub, personal communication, 2010).

Self-imcompatibility (SI)

Production of hybrid seed of some vegetable crops is carried out through the use of SI genes, including cabbage, broccoli, kohl rabi, and cauliflower. One parent of the hybrid has one set of SI genes and the other parent as another set (Figure 2).

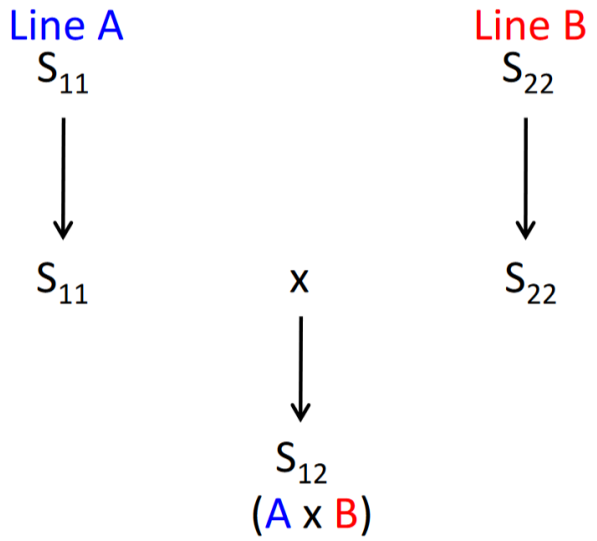

Figure 2. The commercial production of F1 hybrid seed of khol rabi involves the use of two self-compatible but cross-compatible lines and insects for pollination. Adapted from Verna and Sharma (2004).

The principles of the SI system of any crop are described in detail by Kalia and Sharma (2004) with broccoli. The primary method used to overcome SI for inbreeding is bud pollination. Bud pollination involves the application of pollen from a plant on the stigma of a flower of the same plant before the SI mechanism is activated.

One limitation of the SI system for hybrid seed production is the production of enough self-pollinated seed of each parent for planting a hybrid seed production field. Another limitation of the system is obtaining inbred lines with stable SI so that self-pollinated seed is not produced during hybrid seed production. These problems with the SI system have restricted its use to certain vegetable species for which the costs of implementing the system can be covered by the value of the hybrid seed.

Cytoplasmic-genetic male sterility

The cytoplasmic-genetic male sterility (CMS) system makes it possible to obtain female plants in a hybrid seed production field without the roguing required when genetic male sterility is used alone. CMS is used extensively in species with perfect flowers and in some species with monoecious flowers. The system involves an A-line that is the male-sterile female parent used in the hybrid seed production field, the B-line is the maintainer used to produce seed of the A-line, and the R-line (restorer) is the male parent used in the hybrid seed production field.

The unique aspect of the CMS system is the use of a cytoplasm that renders the plant male sterile, unless the nucleus has genetic factors in the nucleus that overcome the cytoplasmic factors and restore male fertility. The cytoplasm of an individual is transmitted to its seed through the egg cell and not through the pollen.

To develop an elite A-line for the CMS system, it is impossible to cross an A-line to another A-line to develop a segregating population because both parents would be male sterile and no seed would be produced. Therefore, the breeder first must develop a superior male-fertile B-line with normal cytoplasm and non-restorer (rf) genes by crossing two or more B-line parents together to obtain a segregating population. The details of how to select a superior B-line from the population is the topic of Lesson 9. A new elite B-line is

used as the recurrent parent in a backcross to develop an A-line that is as genetically identical as possible to itself for all the nuclear genes. Both the B-line and A-line would have non-restorer genes; however, the A-line would be male-sterile because it has the factor in its cytoplasm that renders its pollen male sterile.

A new elite R-line must have dominant nuclear genes (Rf genes) that overcome the sterile cytoplasm of the A-line, resulting in a male-fertile hybrid for the farmer. To develop a segregating population for selection, R-line parents are crossed to each other to assure that individuals in the population will have the necessary restorer genes. After an elite R-line is identified, it can be used directly as the male parent for hybrid seed production.

Seed of the A line is produced by crossing it to its B-line maintainer. For a crop such as maize, the A-line and B-line are planted in strips similar to that illustrated in figure 7. The row of the B-line is destroyed after pollination to avoid accidentally harvesting it. Seed of the B-line and R-line are produced by planting each of them in isolation and letting them naturally pollinate.

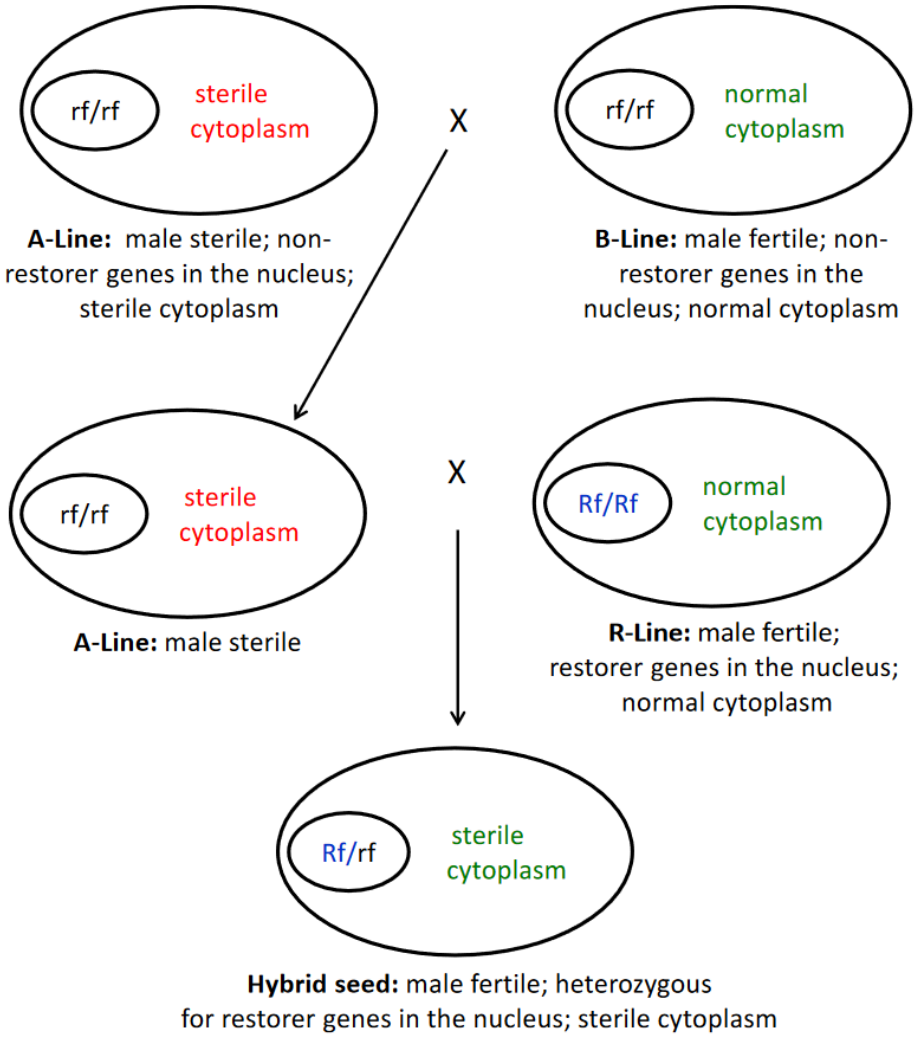

Figure 3. The CMS system used for hybrid seed production. Rf refers to fertility restorer genes in the nucleus and rf refers to nuclear genes that cannot restore the fertility of a plant with sterile cytoplasm.

Transgenic methods for the development of male sterile parents

The SeedLink Invigor system

A gene named *barnase* from the soil bacterium called *Bacillus amyloliquefaciens* makes a defense protein that destroys RNA molecules to kill potential enemies. The bacterium also produces another protein called *barstar* that it uses to protect itself from the toxicity of barnase [Nature. 347:737-741 (1990)]; [Nature. 357:384-387 (1992].

Scientists at Bayer CropScience developed the SeedLink Invigor system for canola hybrid seed production that utilizes the *barnase* (BS) and *barstar* (BR) genes linked with the *bar* (L) gene for glufosinate (Liberty) tolerance. More information about the genetic elements used in the SeedLink Invigor system can be found on pages 9, 11 and 12 of Bayer CropScience application for deregulation of the system in Europe: MS8/RF3 Request for Renewal [PDF]. The principle components of the system are illustrated in figure 2, and explained in greater detail in the figure below. .

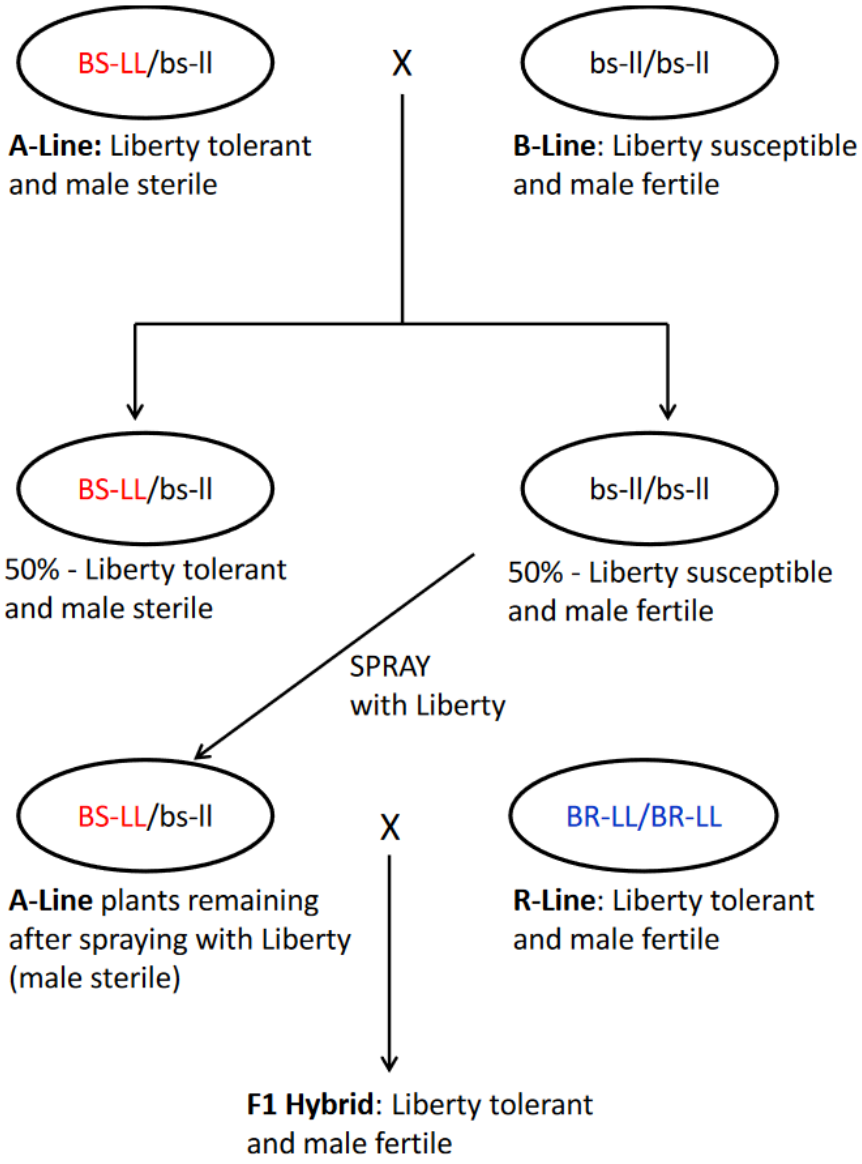

Figure 4. A schematic of the SeedLink Invigor system used for canola hybrid seed production. BS represents the barnase gene that results in sterile pollen, L represents the bar gene for glufosinate (Liberty) tolerance, and BR represents the barstar gene that restores the male fertility of plants with the BS gene.

The following is an explanation of figure 4 and describes how the SeedLink Invigor system is implemented by a canola breeder.

Development of the A- and B-lines

Step 1. The breeder develops new B-lines from breeding populations formed by crossing two or more B-line parents to each other, the same as for the CMS and SPT systems. The populations and elite B-lines derived from them lack the transgenes associated with the Invigor system.

Step 2. An A line is developed that is genetically identical to the B-line, but that has the BS-LL transgene.

This is accomplished by crossing an elite B-line to plants of an available A-line that are heterozygous for the transgene. Because the A-line is heterozygous, 50% of the F_1 plants from the cross will be heterozygous for the transgene and 50% will be homozygous for absence of the transgene. The F_1 plants are sprayed with Liberty herbicide to kill those that lack the transgene. The heterozygous male-sterile F_1 plants are backcrossed to the B-line that serves as the recurrent parent. The BC_1F_1 plants will segregate for the transgene in the same ratio as the F_1 plants. When they are sprayed with Liberty, the surviving heterozygous male-sterile BC_1F_1 plants will be crossed to the B-line to produce BC_2F_1 seed. This process will continue for as many backcrosses as desired to obtain an A-line and its B-line maintainer that are genetically the same, except for the BS-LL transgene.

To produce seed of the A-line for planting a hybrid seed production field, the A-line is planted in alternating strips with its B- line. The A-line will segregate with 50% of the plants containing the BS-LL transgene and 50% lacking the transgene. The A-line strips are sprayed up to three times before pollination with glufosinate herbicide to kill the plants lacking the BS-LL transgene. At maturity, the A-line strips are harvested.

To obtain seed of the B-line that is needed for producing seed of the A-line, it is planted in an isolated field that is not sprayed with glufosinate.

The R-lines used as the male parent for hybrid seed production can be developed from populations lacking the BR-LL transgene or from populations in which one or both parents have the transgene. If the elite R-line lacks the transgene, it must be incorporated by backcrossing. When backcrossing is completed, seed of the R-line is produced in an isolated field for use in a hybrid seed production.

The hybrid seed production field will have strips of the A-line and R-line. The A-line will be sprayed at least three times with glufosinate to kill the 50% of the plants that lack the BS-LL transgene and are male-fertile. The remaining plants are heterozygous for the transgene and male-sterile. The R-line is homogeneous for the BR-LL transgene and male-fertile. None of the plants in the R-line will die if sprayed with Liberty. The seed harvested from the A-line is the hybrid seed that will be sold to the farmer. The hybrid plants grown by the farmer will have the genotype BR-LL/br-LL and BS-LL/bs-LL, which makes them tolerant of glufosinate and male-fertile because the BR gene is dominant to the BS gene.

The Seed Production Technology (SPT) system

The SPT system of Dupont/Pioneer is an alternative to the CMS system for producing hybrid seed corn without detasseling. One weakness of the CMS system in corn is the possibility that the cytoplasmic factors that cause male sterility could make the hybrid plants in a farmer's field susceptible to a disease, as occurred in the 1970's with the Southern corn leaf blight.

The SPT system involves a transgene the *DsRed2* (*Alt1*) gene, designated "F" in this description, that produces a fluorescent pinkish-red color in the seed; the wild-type nuclear male-fertility *Ms45* gene, designated "Ms", required for the production of fertile pollen; and the *zm-aa1* gene, designated "A", which produces the α-amylase enzyme that destroys pollen by breaking down starch in the pollen, but does not reduce the viability of egg cells. A description of the system can be downloaded for review: Petition for the Determination of Nonregulated Status for Maize 32138 SPT Maintainer [PDF].

The following discussion explains how the system is implemented by a corn breeder who is responsible for developing new elite inbred lines.

Development of the A- and B-lines

Step 1. The breeder develops elite B-lines from breeding populations formed by crossing two or more B-lines to each other, the same as for the CMS and InVigor systems. The populations and elite B-lines derived from them lack the transgene associated with the SPT system and have the dominant Ms allele for male fertility that is present in essentially all of the corn germplasm used by breeders for inbred line development.

Step 2. An elite B-line that lacks the transgene and has the wild-type Ms allele will have the genotype f-ms-a/f-ms-a Ms/Ms. The f-ms-a/f-ms-a indicates that the transgene is absent. The B-line must be converted into two genetic types by backcrossing: an A-line and a B-line maintainer. The process begins by crossing the new B-line as male to an existing B-line maintainer that is hemizygous for the transgene and homozygous for the recessive ms allele (F-Ms-A/ f-ms-a ms/ms). The recessive ms gene that causes male sterility in the homozygous condition was found in the maize germplasm collection and is not a transgene. The transgene and ms gene are independently inherited and both can be detected in a plant by the use of perfect molecular markers for each. The F_1 seed from the cross will have the genotypes F-Ms-A/f-ms-a Ms/ms or f-ms-a/f-ms-a Ms/ms, both of which will be male fertile because they have the dominant Ms allele. The seeds with the genotype F-Ms-A/ f-ms-a Ms/ms are selected based on their fluorescent red color. The F_1 plants from the selected seeds are backcrossed to the elite B-line to obtain the BC_1F_1 seeds, which have the genotype F-Ms-A/f-ms-a Ms/Ms, F-Ms-A/f-ms-a Ms/ms, f-ms-a/f-ms-a Ms/Ms, or f-ms-a/f-ms-a Ms/ms. The seeds with the genotype F-Ms-A/ f-ms-a Ms/ms are selected based on their fluorescent red color and by the use of a molecular marker that can identify heterozygous Ms/ms seeds. In each subsequent backcross generation, the selected seeds are planted and the $BCxF_1$ plants are backcrossed to the elite B-line. When a sufficient number of backcrosses have been made, the $BCxF_1$ plants with the genotype F-Ms-A/- Ms/ms are self-pollinated to recover F_2 seeds with the genotype F-Ms-A/ f-ms-a ms/ms to use as the B-line maintainer and F_2 seeds with the genotype f-ms-a/f-ms-a ms/ms to use as the A-line. Because the A-line and B-line are derived from the same $BCxF_1$ plants, they will be genetically identical for all of the nuclear genes, except for the transgene.

Development of the R-lines

The R-lines are developed from breeding populations formed by crossing one or more R-lines to each other. The populations and the R-lines selected from them will have the wild-type Ms allele that is in a conventional corn germplasm. The new R-line can be used as is for hybrid seed production without additional breeding.

Production of A-line seed

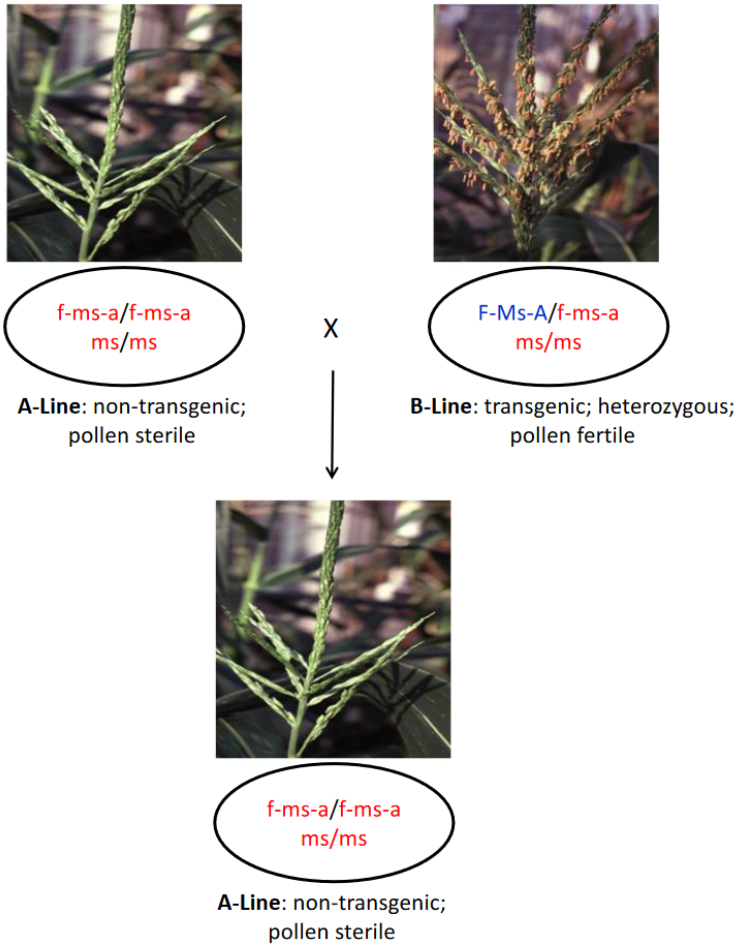

Figure 5. The A-line and B-line are planted in strips (figure 4). Half of the B-line pollen has the genotype F-Ms-A ms and is not viable because it has the A gene that destroys the starch. The other half of the pollen with the genotype f-ms-a ms is viable and fertilizes the A-line whose eggs have the genotype f-ms-a ms. As a result, all of the seed of the A line lacks the transgene and is homozygous for the ms/ms allele. When the A-line seed is planted as the female parent in a hybrid seed production field, all of the plants will be male sterile.

Production of B-line seed

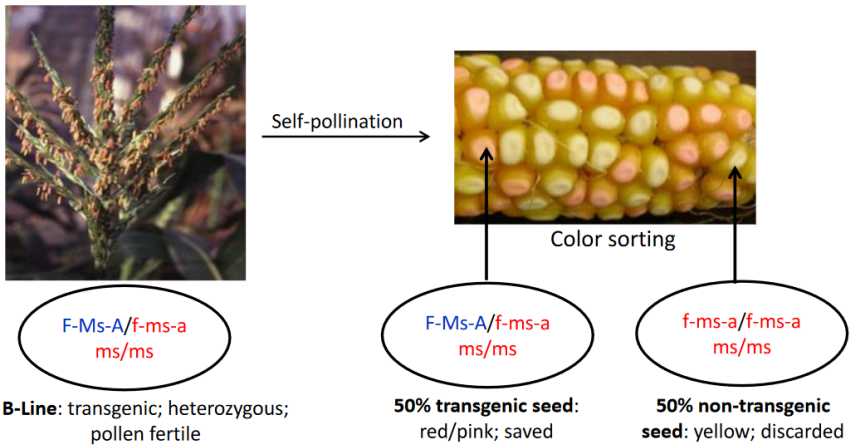

Figure 6. Seed for the B-line is produced by planting it in an isolated field and letting it naturally pollinate itself. The half of the harvested seed that is hemizygous for the transgene will be fluorescent red and genotypes of the other half without the transgene will be yellow. The seed is sorted electronically. The fluorescent red seeds are saved for planting the maintainer line again and the yellow seeds without the transgene are discarded.

Production of R-line seed

R-line seed is produced by planting it in isolation and letting it naturally pollinate. All of the seed will lack the transgene and be homozygous for the wild-type Ms allele, which makes the R-line male fertile.

Production of hybrid seed

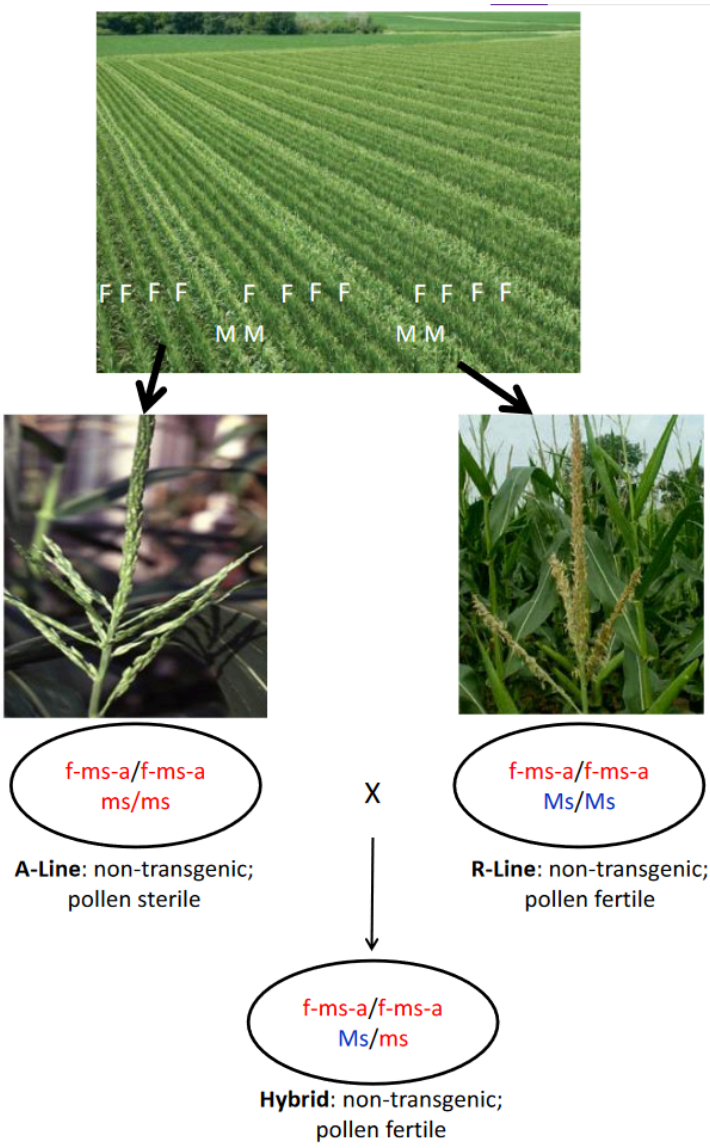

Figure 7. For hybrid seed production, the A-line is pollinated by an R-line. Both the A- and R-lines are non-transgenic. The A-line is homozygous and homogeneous for a nuclear ms gene that makes it male sterile and the R-line is homozygous and homogeneous for the wild-type MS allele. The field design is shown above and depicts four rows of the female parent (A-line) alternating with two rows of the male parent (R-line). The F_1 seed sold to farmers will be male fertile and lack the F-Ms-A transgene.

Chemical hybridizing agents (CHA)

The use of CHA to obtain male-sterility of the female parent in a hybrid seed production system has the potential advantage of eliminating the need for a breeder to incorporate specific genetic factors for male-sterility into inbred lines. They have been extensively studied in wheat in comparison with the use of CMS. The studies of Cisar and Cooper (2002) and Adugna (2005) are just two of many that have been published

on the subject. Three of the most important attributes of a CHA are that they should cause male sterility without reducing female fertility, the male sterility should be complete, and it should be effective on a range of genotypes. A few CHA have been found that meet most of these attributes and have been used to produce commercial hybrid seed of a few crops that have had difficulty finding reliable CMS systems, such as wheat (Cisar and Cooper, 2002).

Requirement 3: *Transfer of pollen from the male to the female parent*

The most cost-effective method of transferring pollen from the male to the female parent is by wind or insects. When insect pollination is involved, insects commonly are brought to a commercial field to assure that an adequate number are present, such as in alfalfa, sunflower and canola.

Figure 8. The commercial production of alfalfa involves the use of leafcutter bees for pollination. Structures in the middle of the field are leafcutter beehives. Photo courtesy of Charles Brummer, Noble Foundation.

Manual pollination for the production of hybrid seed is done for some species with perfect flowers, such as tomato.

Effective pollen transfer has prevented the use of hybrid cultivars in some self-pollinated species, such as soybean. Although some pollen transfer occurs by insects, the amount of hybrid seed production is too low and unreliable for large-scale production. In wheat, the range of seed set through wind pollination ranges from 10 to 70%, which has limited the use of hybrid cultivars for commercial production (Adugna, 2005).

Requirement 4: *Reliable and economical hybrid seed production*

The ultimate requirement for hybrid cultivars is that they be profitable for the breeder, the company producing the commercial seed, and the end users. In order for this to occur, the hybrid cultivar must perform better than the other cultivar types of the species to offset the greater seed cost. The greater

seed costs are associated with more expense for breeding the parents of the hybrid and the greater cost of producing the hybrid seed.

Applied Learning Activity 8

Breeding research has been done for at least 50 years to develop hybrid cultivars of wheat, yet the area planted to hybrid cultivars is far less than that of pure-line cultivars. Your assignment is to use resources available through the internet to write a report that addresses the following questions. The report should be not more than three pages long, single spaced, with a 12-point font. Cite the references used for the information in the same style as used for research articles in *Crop Science*.

1. How much high-parent heterosis is there in wheat?

2. For the hybrid cultivars that have been grown commercially, what methods have been used to eliminate fertile pollen from the female parent?

3. How has pollination been carried out to obtain adequate seed set on the female parent?

4. What have been the primary factors limiting the widespread use of hybrid wheat cultivars?

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Hybrid Cultivars

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 34 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)

There are four steps in the development of a hybrid cultivar: the development of populations for selection, the development of inbred lines, testing of inbred lines for combining ability, and the production of commercial hybrid seed.

Step 1: *Development of populations for selection*

The types of crosses made to develop inbred lines for hybrid cultivars are similar to those used for the development of pure-line cultivars. Single-crosses between elite inbreds are the most popular, although other types of crosses such as the three-way or backcross are also used when appropriate. However, a major difference in population development for the two types of cultivars is the importance of heterotic groups for hybrid cultivars. The breeder of a hybrid cultivar will carry out an independent program for inbred line development for each heterotic group. For example, in maize a breeder will develop inbred lines related to the Stiff Stalk Synthetic and independently develop inbred lines related to non-Stiff Stalk germplasm. Crosses between inbred lines of the two heterotic groups are avoided for population development. The goal is to have elite inbred lines from each heterotic group that will produce a superior hybrid when crossed together.

The use of cytoplasmic-genetic male sterility (CMS) for production of commercial hybrid seed requires one breeding program for the development of superior A- and B-lines and another for the development of superior R-lines. The A- and B-line germplasm can be considered one heterotic group and the R-line germplasm another heterotic group.

For development of populations for selection of improved A-lines, it is impossible to cross two A-lines because they are male sterile. Therefore, male-fertile B-lines are crossed to form the breeding populations. Independently, crosses are made between elite R-lines to develop populations for selection of improved R-

lines. Crosses between B- lines and R-lines are not made because it disrupts the heterotic groups and results in segregation of restorer and non-restorer genes that is undesirable.

Step 2: *Development of inbred lines*

The methods available to develop inbred lines for hybrids are the same as for those developing pure-line cultivars. Review the methods of generation advance that were discussed in the inbreeding chapter of *Plant Breeding Methods* for more information.

The only method that may be carried out differently for the development of inbreds lines compared with pure-line cultivars is early generation testing. Early generation testing for inbred line development can involve the evaluation of the performance of lines *per se* prior during the inbreeding process. Such tests are for traits that an inbred line must have in order to be as useful parent of a hybrid. For example, if disease resistance is required in a hybrid, the breeder may select for resistance in the inbreds. This selection may be carried out among lines in an early generation, such as $F_{2:3}$ or $F_{3:4}$ lines. The desirable lines are further inbred before they are considered adequately homozygous and homogeneous for used in a commercial hybrid.

The yield of inbred lines *per se* generally is not considered a good indication of their combining ability for the trait. Early generation testing for yield involves crossing a individuals (plants or lines) to a tester from the other heterotic group and evaluating the performance of the testcross seed. Individuals with superior testcross performance are saved for continued inbreeding and the inferior ones are discarded.

When early generation testing is used by a breeder, inbreeding and testing generally are carried out simultaneously to reduce the number of years required for developing a finished inbred. For example, a breeder may grow $F_{2:3}$ lines from a population in Iowa, select one or more F_3 plants from lines with desirable traits, and cross the line to a tester from another heterotic group. During the winter, the F3:4 lines would be grown in Hawaii where it is not possible to select for traits of interest. The breeder would harvest one or more random F_4 plants from each line. During the following summer in Iowa, the testcross seed of each $F_{2:3}$ line would be evaluated at multiple locations for yield and the $F_{4:5}$ lines that trace to each of the $F_{2:3}$ lines would be grown. The $F_{4:5}$ lines would be evaluated for traits required of the inbred *per se* and individual F_5 plants would be harvested from each of the selected lines. After the yield data were available from the testcross, any F_5 plants that trace to $F_{2:3}$ lines with superior performance would be advanced to Hawaii for additional breeding and those from $F_{2:3}$ lines with inferior performance would be discarded.

A breeder is faced with finding a balance between the expected traits of the inbred *per se* and its combining ability. One notable example involved the development of B73, an inbred line of maize related to the Stiff Stalk Synthetic that was extensively used for production of hybrid cultivars for many years and was an outstanding parent for the subsequent development of populations from which superior progeny were selected. Dr. Wilbert Russell, a maize breeder at Iowa State University, grew a yield test of lines with testcross seed and planted the lines *per se* at Ames, IA. During the summer, he selfed plants of the lines and evaluated them for traits he considered important to have in a finished inbred. He discarded the line that became B73 because he believed its traits would not be good enough to use it for producing commercial hybrids. When the data from the yield test were available, he found that the yield of the line in testcrosses

was outstanding i.e. the line had outstanding combining ability when crossed to a tester from the non-Stiff Stalk group. Fortunately, Dr. Russell had saved seed of the line in storage as a precaution. Based on the testcross results, he used that seed from storage to continue to inbreed and test the line that became B73.

The use of early generation testing for the identifying superior B- lines or R-lines with good combining ability requires a way of obtaining testcross seed. For R-line development, testcross seed is produced by crossing the experimental R-lines to an elite male-sterile A-line tester. The absence of fertile pollen in the female A-line tester facilitates crossing, even when it is done by hand. Unfortunately, obtaining testcross seed for B-lines is more difficult because both the B-line and the R-line tester are male fertile. It is not practical in many species with perfect flowers to obtain enough testcross seed by hand emasculating the male-fertile B-line or R-line.

One method is to obtain testcross seed for evaluating the combining ability of B-lines is to treat them with a chemical hybridizing agent, such as is done in wheat. Another alternative is to convert the B-lines to their A-line counterparts during the inbreeding process, as describe for sorghum on pages 436 and 437 of *Principles of Cultivar Development. Vol. 1.*

The following video presentation by Sheilah Oltmans-Deardoff describes breeding and the commercial production of hybrid seed for canola: Hybrid Production Video

Step 3: *Testing of inbred lines for combining ability*

The ultimate test of the value of an inbred line is its ability to produce a superior hybrid when crossed to another inbred line. There are some breeders who prefer to use early-generation testing during the inbreeding process to select lines with the greatest potential for superior specific combining ability. The disadvantage of the method is that resources must be used for testing unfinished lines that could be spent on testing finished lines. The advantage of early generation testing is that a greater percentage of the finished lines should have good combining ability compared with those obtained without early generation testing. There are other breeders who prefer to develop finished lines through single-seed descent, pedigree, doubled haploids, or another method without early-generation testing. The advantage and disadvantages of this strategy are the opposite of those for early-generation testing.

Regardless of which of the two strategies the breeder utilizes, the finished inbred lines must undergo extensive testing in multiple locations and years to find those that will produce a superior commercial hybrid. The first stage in the process is to eliminate inferior lines from further consideration. For this purpose, the breeder will mate each line to one or a few inbred testers from another heterotic group. The testers generally are elite inbred lines currently used in commercial hybrids. The test can be considered an evaluation for both general and specific combining ability. It can be considered a test of general combining ability because research has shown that lines that perform well with one tester are likely to perform well with other testers. It is a test for specific combining ability if the experimental line ultimately is used with the same tester to produce a commercial hybrid. For example, the inbred line of maize B73 was commonly used as a tester for evaluating the combining ability of lines from the non-Stiff Stalk group. After the initial test, selected lines were crossed to other elite inbreds from the Stiff Stalk group to find those inbred

combinations that would result in a new commercial hybrid. In some cases, selected lines were crossed to B73 itself to produce a commercial hybrid.

The first yield evaluation of the inbred lines generally is done at limited number of locations in one year. It is common to use only one replication at each location because it provides more information about the performance of a line across environments than if replicated plots are used in fewer environments. This principle was covered in Lessons 1 through 3 at the beginning of this course.

In the second stage of testing, the number of lines is reduced from that of the first year, the number of testers is increased, and the number of locations of testing is greater. The breeder places more emphasis on obtaining data from as many locations as possible, than on replication of the entries in the test.

For each subsequent year of testing, the number of lines continues to be reduced and the number of testers and locations of testing increases. At the final stages of testing a potential new hybrid, it is common to use strip tests planted and harvested by farmers to obtain data from a larger number of environments than would be possible for the breeder to conduct.

Step 4: *Production of commercial hybrid seed*

Seed of an inbred line that is selected for use in a hybrid must be purified and increased. The factors that must be considered in the timing of the process relative to testing of the line are the same as those for any of the other cultivar types. It is less expensive to delay the seed increase phase until the final decision is made to use the line to produce a commercial hybrid; however, this delays the availability of the hybrid seed for the end user.

The options for purifying and increasing seed of an inbred line are the same as those discussed for pure-line cultivars in Lesson 7. A high level of uniformity generally is required for inbred lines so that off-type plants can be readily identified and removed in a field used for hybrid seed production. For this reason, progeny testing of individual plants of a line is a commonly done to obtain pure seed.

When CMS is used to produce hybrid seed, B lines must be converted to their A line counterpart. This generally is done by backcrossing. The A line is used as the female donor parent and the B line is the recurrent parent. All of the offspring in each backcross should be male sterile. One of the key factors that the breeder will evaluate during the backcross process is whether the backcross progeny are completely male sterile. If they are not, the B line and the backcross progeny must be discarded because the A line counterpart will not be completely sterile in a seed production field, which would not be acceptable. The evaluation for sterility is one of the steps described for sorghum on pages 436 and 437 of the text.

Maize and canola will be used as examples of crop species for which hybrid cultivars are grown commercially.

Example 1: Maize

Maize grown commercially in the United States is produced from hybrid seed derived from crosses between inbred lines. Inbreds used as females are selected based on their yield of high quality seed, good silk

production, and good plant health. The inbred used as the male parent must have good pollen production and good plant health. The female inbred line has to be rendered incapable to self-pollinate. One method is by physical removal of its tassel containing the male flowers, referred to as detasseling. Detasseling can be done manually, mechanically, or both. The use of manual labor and mechanization for female detasseling in maize is shown in this YouTube video: Detasseling Video

Another important aspect in hybrid maize seed production is the isolation of the female parent from other sources of pollen to maintain the genetic purity of the hybrid seed (figure 1). Effective isolation requires leaving a sufficient distance between the field used for seed production and other maize fields. For the state of Washington, the isolation distance for hybrid maize is 415 feet.

Hybrid seed production in corn involves planting the female and male parents in strips. The most common planting pattern is fours of the female parent and one row of the male parent. At the time of flowering, there are three methods used to eliminate fertile pollen from the female parent. (1) The female parent can be detasseled by hand, machine, or both. (2) In the cytoplasmic male sterility (CMS) system, the female parent is the male-sterile A line and the male parent is the male-fertile R-line. (3) For the SPT system of Pioneer Hi-Bred, the female parent is homozygous and homogeneous for a nuclear ms gene that makes it male sterile and the male parent is homozygous and homogeneous for the wild-type MS allele. The following photos were provided by Walter R. Fehr, Iowa State University.

1. Regardless of the system used to eliminate fertile pollen from the female parent, the row of the male parent is cut down after pollination is complete to assure that none of its seed is accidentally mixed with the seed of the female parent at harvest time.

2. The female rows in this field were detasseled with a machine that cut off the tassel, as illustrated in the following slide.

3. After the machine has cut off the tassel of the female rows (F), persons walk the rows to remove any tassels that were missed.

4. After flowering is complete, the male parent is cut down to avoid accidentally harvesting its seed when the hybrid seed is harvested from the female parent.

5. The harvester is removing hybrid seed corn near Boone, IA. The entire ear, including the husks, are picked and elevated into a wagon in the back of the machine. The husks serve to protect the seed during the harvest operation.

6. A harvester with a wagon behind it.

7. A tractor takes an empty wagon to the field to get the ears that have been harvested.

8. The wagon trailing the harvester is unloaded into the wagon behind the tractor.

9. The wagon behind the tractor also can be filled directly from the harvester.

10. The loaded wagon is taken from the field as the harvester continues through the field.

11. The loaded wagons are emptied into a semi-trailer that will transport the ears to the processing facility.

12. The ears are dumped from the wagon into the semi-trailer.

13. The loaded truck hauls its load to the processing facility. At the processing facility, the trucks are weighed before unloading.

14. The trucks are unloaded onto a conveyor belt. The facility is able to unload trucks with different hybrids at the same time.

15. The ears are conveyed to a building were the husks are removed and the ears inspected to be sure the seed does not have mold or other diseases that could make the seed unhealthy.

After the ears are inspected, they are conveyed to a building where the seed is dried while still on the cob. Drying is necessary because the ears are harvested at relatively high moisture to minimize damage to the seed during harvest and transport. After the seed is dry enough, it is removed from the cob, conditioned, sized, treated, bagged, and stored until it is delivered to the farmer for planting.

Example 2: Canola

Production of commercial hybrid seed for canola relies on male sterility systems. Seed production fields are designed to contain alternating strips of male and female (about 8 male: 24 female) rows. Each acre will have about two beehives housing alfalfa leaf-cutter bees for pollination of the female parent (Figure 16). The isolation distance for canola hybrid seed production is 800 m.[1]

1. See the "Inspection Requirements" section on the Canadian Food Inspection Agency's website for canola seed crop inspection.

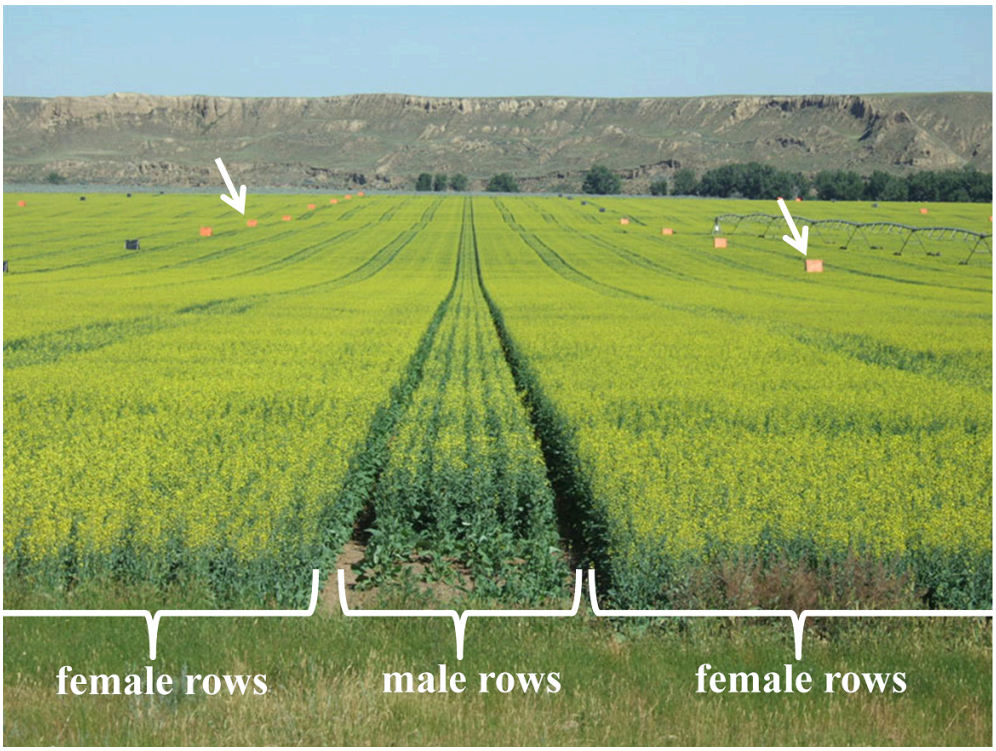

Figure 16. Canola seed production field. The male sterile line (female rows) is interspaced with male rows. Hives of the alfalfa leafcutter bees (orange objects pointed by the arrows) are placed in various locations within the field. For the production of A-line seed the same field arrangement will be used. The female line (A-line) will be pollinated by the male line (R-line). Photo courtesy of Shui-zhang Fei, Iowa State University.

After pollination rows of male parent are removed to prevent contamination of hybrid seed produced in the female parent with the seed from self-pollination produced in the male parent (Figure 17).

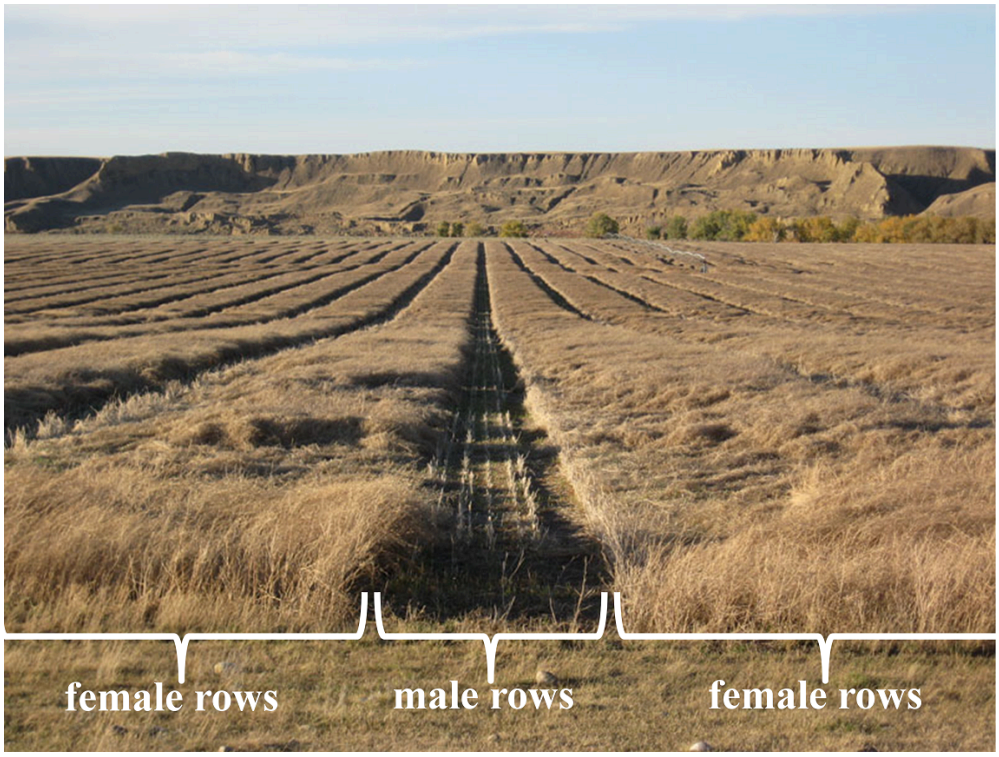

Figure 17. The male rows used for pollination (figure 17) were removed after pollination. Photo courtesy of Shui-zhang Fei, Iowa State University.

Applied Learning Activity 9

It is your responsibility to develop improved single-cross hybrids of rice for Arkansas through the use of cytoplasmic-genetic male sterility (CMS). You can grow one crop per year in Arkansas and another crop during the off-season in Brazil. In Arkansas, you can conduct all of the necessary breeding activities. In Brazil, you can do crossing, inbreeding, and production of testcross seed, but not selection or yield evaluation.

For the development of R-lines derived in the S4 generation, compare (1) single-seed descent and (2) early generation testing.

1. Outline season-by-season your strategy for each method. Begin by developing single-cross breeding populations in Brazil. For early-generation testing, evaluate testcross seed of S1:2 lines for one year with as many locations and replications as you consider appropriate. Be sure to use every available season for inbreeding when doing early-generation testing so that you have S4-derived lines ready as soon as you do by single-seed descent. What type of tester and how many will you use for the early-generation test. End your answer when you have seed of finished S4-derived lines ready for evaluation.

2. Which of the two methods would you prefer to use and why? Be sure to take into consideration the cost of obtaining S4-derived lines by each method.

Applied Learning Activity 10

1. You are developing improved R-lines of sorghum for use in a single-cross hybrid. You will conduct three years of yield evaluation of S6-derived lines to select one to use in a commercial hybrid. You have one self-propelled plot combine that can harvest 20,000 yield test plots each year for all phases of yield testing. Outline how you would carry out the three years of testing. For each year, indicate how many lines you are testing, the type of tester (R-line, B-line, A-line), how many testers you would use, the number of locations of testing, the number of replications at each location, and the number of plots used. **The total number of plots across the three years must not exceed 20,000 i.e., Year 1 plots + Year 2 plots + Year 3 plots must not exceed 20,000.**

    a. Year 1:

    b. Year 2:

    c. Year 3:

2. You have identified an S6-derived B-line that you want to convert to an A-line. Outline season-by-season how you would develop a BC3-derived A-line as genetically similar to the B-line as possible, except for CMS.

3. Describe your procedure for producing breeder seed of an R-line and an A-line.

4. Describe the fields that must be grown each year to produce commercial single-cross hybrid seed with CMS to sell to the farmer.

References

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Synthetic Cultivars

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 33 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)

In the United States, the primary use of synthetic cultivars is for forage and turfgrass species. In other parts of the world, synthetic cultivars, sometimes referred to as open-pollinated cultivars, are used for additional crops such as maize. The focus of this chapter will be on forage and turfgrass species.

Forage and turfgrass species have some common characteristics that are important for breeding synthetic cultivars.

1. Although they have perfect flowers, self-pollination is minimized because of self-incompatibility.
2. Synthetic cultivars are highly heterozygous and heterogeneous. Inbreeding depression is severe and plants that develop from self-pollinated seed lack the vigor of those obtained by cross-pollination.
3. In a heterogeneous population, each plant is genetically different from another. Although it is appropriate to refer to each individual plant as a genotype, it is common to refer to each plant as a clone because it is possible to propagate individual plants vegetatively.
4. The species are perennials.

There are four steps in the development of a synthetic cultivar.

Step 1: *Develop a segregating population for selection*

There are two common types of populations used for selection.

- (1) Synthetic cultivars *per se* can be used as populations because they are heterozygous and heterogeneous. One example was the use of the synthetic cultivar Freedom red clover as the

population for selection of the cultivar Freedom MR! [JPR 2:205–207 (2008)].

Populations are formed by intermating clones from different sources. The intermating is commonly done by planting the clones in a polycross and allowing them to cross naturally by wind or insects. The parent clones are selected based on the traits that the breeder would like to have in a new cultivar. The parent clones commonly differ in the traits that they contribute to the population, which makes it possible to select for multiple traits among the progeny. For example, the synthetic cultivar Recovery wheatgrass [JPR 5: 367–373 (2011)] was derived from three parent clones, Rosana, D2945, and WW117FC that differed in vigor, seedling establishment, sod-forming ability, and forage and seed production.

Step 2: *Selection of superior clones*

Selection begins as soon as seed of the segregating population is available. There is no inbreeding of a population because of severe inbreeding depression.

The first evaluation of individual clones generally is done by phenotypic selection. A breeder may evaluate thousands of plants for traits of importance. If selection is practiced for disease or insect resistance, artificial infection commonly is done in a controlled space, such as a greenhouse or growth chamber. The breeder may select in a stepwise manner to improve the efficiency of the process. For example, selection may be practiced for resistance to one disease, following which the selected individuals are exposed to a second disease. After the second selection, the chosen clones may be vegetatively propagated to the field where they are evaluated for response to environmental factors, such as winter hardiness and plant vigor.

It is common for breeders to use recurrent phenotypic selection for one or more cycles to improve the population for traits of interest. The improved population *per se* may become a new synthetic cultivar. For example, the synthetic cultivar TifQuik bahiagrass [JPR 5:147–150 (2011)] was a result of four cycles of selection for fast germination from the cultivar Tifton.

If genotypic selection is used to identify superior clones, it generally is done after phenotypic selection has been used to select clones for traits of interest in a new cultivar. Genotypic selection is done to determine the general combining ability of clones for traits that are considered difficult to assess on a phenotypic basis, such as yield. The clones to be evaluated are grown in a polycross to obtain half-sib seed. The half-sib seed is harvested from each clone and planted in replicated tests in multiple environments. A test at a location generally is evaluated for multiple years under the conditions in which the species is grown commercially. For example, the cultivar Nelson annual ryegrass [JPR 5:45–48 (2011)] was developed for use as a forage crop in the southern USA and field tests for performance were evaluated at Overton and Beaumont, Texas, across 3 years.

When genotypic selection is practiced, the clones under evaluation generally are maintained in the field, commonly referred to as a maintenance nursery. After the superior clones are selected, vegetative propagules from the maintenance nursery are used for production of seed for testing experimental synthetics and seed of the final cultivar.

An example of the process of developing a synthetic cultivar is provided for alfalfa in the following presentation by Sheilah Oltmans-Deardoff:

- Access the slides: Alfalfa-Synthetic-Development [PPT]

Here is another presentation on alfalfa breeding. Photographs were provided by Robert Clark, Forage Genetics International at Boone, IA, and Charles Brummer, The Noble Foundation at Admore, OK:

Figure 1. Seed of an existing cultivar or the hybrid seed obtained by crossing selected individuals is germinated in a greenhouse or growth room to obtain thousands of seedlings, each of which is genetically different. The seedlings (clones) may be evaluated for traits of economic importance in a greenhouse, such as disease resistance, to identify those to be transplanted to the field for further evaluation.

Figure 2. The seedlings are space planted in the field.

Figure 3. Seedlings can be manually transplanted in the field.

Figure 4. The individual clones commonly are evaluated for multiple years to select for ability to survive during the winter (winter hardiness), spring regrowth, forage yield, and resistance to insects and diseases.

Figure 5. Some of the clones do not survive under field conditions. Those that exhibit superior phenotypic traits are selected. Cuttings are taken from the selected clones for use as parents for crossing, such as in a program of recurrent phenotypic selection, or they may be mated in a polycross for genotypic evaluation with half-sib families.

Figure 6. Pieces of the stem of selected clones are harvested in the field, wrapped in a wet towel, and placed in a cooler with ice. The cuttings may be used for manual crossing in a greenhouse or sent to Idaho for planting in a cage where bees are used for crossing.

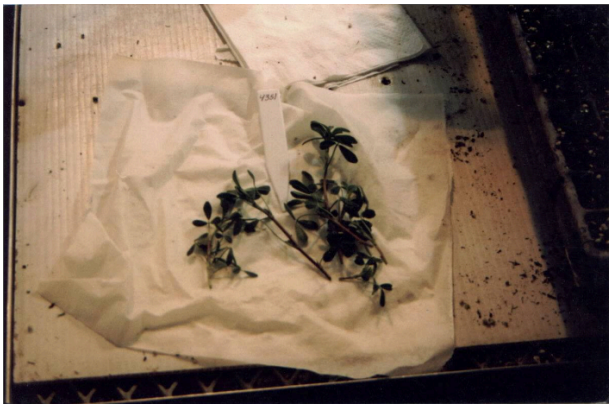

Figure 7. A piece of stem with one node is planted in a rooting media to obtain a seedling.

Figure 8. These persons are preparing stem cuttings taken from the field and placing them in the rooting media.

Figure 9. After the cuttings have developed roots, they are transplanted to pots in the greenhouse for manual crossing or to cages in Idaho for crossing with bees.

Figure 10. These persons are making a polycross of the clones in pots around the room. Pollen is manually transferred among the clones. Note that the persons are sitting on stools so it is easy to move from one plant to another.

Figure 11. For conducting a manual polycross, pollen is transferred among the parent clones by tripping their flowers with a folded piece of paper. Note the pollen on the tip of the paper. The pollen of different plants is mixed together as the flowers are tripped; and as a flower is tripped, the mixed pollen sticks to the stigma, which leads to fertilization.

Figure 12. The pollinated flowers are identified with a tag. The person is holding a mature pod that contains hybrid seed. Each of the seeds in a pod will be genetically different because the pollen is heterogeneous. The seed from each plant is harvested separately. The seed from the parent clones can be mixed together to form a segregating population for further selection or it can be kept separate for evaluating the parent clone genotypically as a half-sib family.

Figure 13. The alternative to manual crossing is to plant the parent clones in a cage where bees are used for crossing. The polycross may be used to produce Syn.1 seed of an experimental synthetic. There can be as many as 200 different experimental synthetics evaluated in a year.

Figure 13B.

Figure 14. The experimental synthetics generally are evaluated with Syn.1 or Syn. 2 seed in multiple locations during several years.

Figure 15. The field trials are sown with a self-propelled planter.

Figure 16. Powered equipment may be used for planting field trials. Photo courtesy of Charles Brummer, The Noble Foundation.

Figure 17. A typical design for alfalfa yield trial.

Figure 18. The half-sib families or experimental synthetics are evaluated visually for disease and insect resistance, winter hardiness, regrowth after cutting, and other important traits.

Figure 19. Forage yield is evaluated with a self-propelled harvester. Samples of the forage are taken to the laboratory to evaluate traits such as digestibility.

Figure 20. Alfalfa seed production in Idaho. Structures in the middle of the field are leafcutter beehives. Leafcutter bees are used for pollination.

Step 3 – *Evaluation of experimental synthetics*

The two general types of synthetic cultivars are broad based and narrow based. Broad-based synthetics are based on a larger number of selected clones, while narrow-based synthetics are based on a relatively few selected clones. There is no specific number of clones that define the two types; however, broad-based synthetics generally would have between 20 to 100 parent clones and narrow-based synthetics generally would have less than 20 clones. Broad-based synthetics generally are based on parent clones that are selected only by phenotypic evaluation, while the clones for narrow-based synthetics are based on phenotypic and genotypic evaluation.

After clones are selected in step 2, they are used to make up experimental synthetics that have the potential to be a new cultivar. The experimental synthetics are designed to have the characteristics that are needed in the final cultivar. One experimental synthetic may emphasize the use of parent clones with resistance to certain diseases, while another experimental synthetic may emphasize resistance to other pests. A breeder may choose to use a parent clone in more than one experimental synthetic.

The parent clones per se are referred to as the Syn. 0 generation. The selected clones are planted in a polycross with vegetative propagules often are obtained from plants in the maintenance nursery. The options for design of the polycross, the number of replications, the method of harvesting, and the method of bulking the half-sib seed from the clones to form the Syn. 1 were previously discussed in *Plant Breeding Methods*.

When Syn. 2 seed is used to plant the test of experimental synthetics, the breeder plants the Syn. 1 seed in isolation, allows the plants to intermate naturally, and harvests the Syn. 2 seed in bulk. No attempt is made to harvest seed from Syn. 1 plants individually.

The breeder must decide whether to evaluate an experimental synthetic with Syn. 1 or Syn. 2 seed. The advantage of using the Syn. 1 is that it takes less seasons to produce the seed. The disadvantage of using the Syn. 1 is that it may not be a reliable indicator of the performance of the synthetic when it is sold commercially as Syn. 3 or Syn. 4 seed. The influence of linkage disequilibrium on the performance of a synthetic in the Syn. 1 versus later Syn. generations should be reviewed on pages 56–57 and 103–104 of the text and on pages 22–23 of the Chapter 11 on alfalfa breeding in *Principles of Cultivar Development*, Vol. 2.

Step 4 – *Production of commercial seed*

Based on the performance of the experimental synthetics, the breeder will choose one or more to release as a commercial cultivar. Vegetative propagules of the parent clones from the maintenance nursery will be used to plant a polycross to obtain Syn. 1 seed in the same manner discussed in step 3. The number of replications will be influenced by the amount of Syn. 1 seed that is needed for planting the size of a field that can produce enough Syn. 2 seed. In addition, it is common to produce enough Syn. 1 seed to put in storage for replanting a field for additional Syn. 2 seed, if necessary. Because the plants are perennial, it is possible to harvest Syn. 1 seed from the polycross more than one year.

The Syn. 1 seed is planted in the field with mechanical equipment. It generally is planted in rows so that it can be cultivated for weed control and for irrigation. The plants are cross-pollinated with each other. The Syn. 2 seed is harvested in bulk with a combine, similar to that used for other row crops. Syn. 2 seed can be harvested from the field for more than one year.

To obtain Syn. 3 seed, the Syn. 2 seed is planted and harvested in bulk as described in the previous paragraph. The Syn. 3 commonly is the class of seed sold for commercial plantings. If Syn. 4 seed is required, the Syn. 3 seed is planted and harvested in bulk as described in the previous paragraph.

The seed producer determines the Syn. generations that will be sold commercially, the class of certified seed used for each, and the number of times a field can be harvested for seed. The cultivar descriptions for alfalfa provided in the www site of the National Alfalfa Review Board are a valuable resource for understanding the alternative available for seed production of synthetic cultivars.

The reason for limiting the number of harvests from a field is to avoid a change in the genetic makeup of the plants in a field. The genetic makeup can change if some plants are lost because they cannot compete with other plants in the mixture. It also can change when seed from one harvest falls on the ground and germinates the following season.

Synthetics of maize

The development of synthetic cultivars of maize and other crops differs from that described for turfgrass and forage species because of several important biological differences.

1. It is possible to inbred maize because inbreeding depression is much less than in turfgrass and forage species.

2. There is no self-incompatibility in maize and some self-pollination can occur, even though it is a monoecious species that is wind pollinated.
3. It is an annual crop.

Synthetic cultivars of maize can be developed by any of the methods of recurrent selection discussed in lessons 1 through 3. Commercial seed is produced by growing the cultivar in isolation each year. An example of the development of maize synthetics is the development of the maize population HIS1 that can be found at [JPR 3:10-13 (2009)]

Applied Learning Activity 11

Answer the following questions for the four synthetic cultivars listed below.

- 'Recovery' western wheatgrass – JPR 5: 367-373 (2011)
- 'TifQuik' bahiagrass – JPR 5: 147-150 (2011)
- 'AU Red Ace' red clover – JPR 5: 11-13 (2011)
- 'Nelson' annual ryegrass – JPR 5: 45-48 (2011)

1. What were the parents of the population used for selection?

2. How was the initial population formed for selection?

3. Were phenotypic selection, genotypic selection, or both used to identify the clones used in forming the cultivar?

4. Was recurrent selection practiced? If yes, describe what was done for one cycle of selection and indicate how many cycles of selection were performed?

5. If genotypic selection was used, how many half-sib families were evaluated and how many years were they tested before the clones used in the cultivar were selected?

6. How many clones were used to produce breeder seed of the cultivar?

7. What Syn. generation was or will be sold to the farmer?

8. What type of intellectual property protection has or will be sought for the cultivar?

Applied Learning Activity 12

The following questions pertain to synthetic cultivars of turfgrass and forage species.

1. For an autotetraploid species, what are the possible genotypes that can occur when multiple alleles are present at a locus in a breeding population? Include the names commonly used in genetics to describe each of the genotypes.

2. Assume that you have 95 elite clones available to you with which to initiate a breeding program for a crop species of your choice. How would you use the clones to develop a population in which to do selection without the use of artificial hand pollinations? Begin your answer with the clones growing in the maintenance nursery in the field and end when you have seed of the population in a bag ready to plant.

3. You can develop either a broad-based or narrow-based synthetic. What is an advantage and disadvantage of each type?

4. Outline one cycle of recurrent phenotypic selection beginning with the seed you developed in part 2 above and ending when you have seed of the cycle 1 population ready for planting. Indicate the number of plants you grow initially and the number of plants selected for crossing. Use an article for a synthetic cultivar in the *Journal of Plant Registrations* as a guide for your answer and give the reference citation for the article.

5. Half-sib progeny testing also is used to develop synthetic cultivars. What are the advantages and disadvantages of half-sib testing compared with recurrent phenotypic selection for selection of superior clones? Describe how you would conduct half-sib progeny testing beginning with 60 clones that you selected from a population by phenotypic selection. Begin with the clones in the maintenance nursery and end when you have selected your clones.

6. Assume that you have selected 34 clones to use in a commercial synthetic. Describe how you would produce Syn. 3 seed of the synthetic for commercial sale. Begin with the 34 clones growing in a maintenance nursery and end when you have the Syn. 3 seed ready to sell.

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Multilines and Seed Blends

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 32 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)

Introduction

This chapter discusses the reason for multilines or seed blends and the methods that can be used to make commercial multilines or seed blends. You will investigate further the commercial uses of seed blends by completing the Applied Learning Activity at the end of this section.

A field planted with a blend of alfalfa and winter rye. (Image Source: Will Paddle via Flickr, CC BY 2.0)

For **seed blends** of multiple plant species or multiple genotypes of a species, the components used are those best suited for the intended purpose. For example, a seed blend of Kentucky bluegrass and ryegrass for planting a lawn will involve mixing seed of the best clonal cultivars of Kentucky bluegrass and the best synthetic cultivars of ryegrass for the geographical region of interest. Breeders generally do not breed cultivars specifically for use in a blend. Instead, cultivars are developed to be used individually. The same cultivars can be used in a seed blend. For example, a company may sell a single synthetic cultivar of alfalfa to some farmers or blend the cultivar with a synthetic cultivar of bromegrass to sell to other farmers.

The most recent use of seed blends is in maize for the concept referred to as **refuge-in-a-bag** that you will investigate as part of the Applied Learning Activity. The articles available to the public do not describe the origin of the **transgenic** and **non-transgenic** hybrids that are blended together. According to industry sources, seed companies first develop inbreds for use in non-transgenic hybrids. The transgenes are

backcrossed into the inbreds of a hybrid. Since multiple transgenes are used for insect resistance, some of them can be incorporated in one inbred and others can be incorporated into the other inbred. The inbreds with the transgene derived from the backcross will be near-isogenic lines of the original inbred. Therefore, inbred plants with the transgene should be the same phenotypically as the plants without the transgene. Similarly, the hybrids with and without the transgenes should be phenotypically the same. As a result, a farmer will not be able to see a difference between hybrid plants with or without the transgene, unless the plants without the transgene show symptoms of insect damage.

To make the seed blend sold to farmers for the refuge-in-a-bag, hybrid seed is produced independently with and without the transgenes. After the seed of each is conditioned, it is mixed in the proportion desired by the company.

Applied Learning Activity 13

1. Seed blends of forage species are used for planting pastures and lawns. Visit the website for the Bailey Seed Company: Pasture Grasses. What is the rationale for commercial use of blends for pastures?

2. Visit the website for the Warner Brothers Seed Company. What is the rationale for the blends of different species that they are selling?

3. A seed blend is used in corn hybrids that contain transgenes for insect resistance. Visit the archived website at Farm Industry News regarding single bag refuge seed. What is the rationale for the use of refuge-in-a-bag, instead of the previous requirement of planting a separate refuge in a field of corn?

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Release and Distribution of Cultivars

Walter R. Fehr and Walter P. Suza

Readings:

- Chapter 36 [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr (Access the full book)
- Appendices D, E, F, G, H, and I [PDF], *Principles of Cultivar Development. Vol. 1: Theory and Technique*, by Walter R. Fehr

Making the decision to release a new cultivar

The process for deciding on the release of a new cultivar varies among private companies and public institutions. For public institutions, the process described in Appendices B and C of *Principles of Cultivar Development* has changed since the text was written; therefore, the two sections were excluded from the reading assignment. Today, every public institution has an independent process for handling the release of new cultivars.

The process at Iowa State University (ISU) will be outlined as one example. **Germplasm** developed by breeders at ISU is considered intellectual property of the university. Appendix D provides an example of the type of disclosure form that is sent by the breeder to the university describing the invention of a cultivar. The university passes the disclosure to the Iowa State University Research Foundation (ISURF) which becomes the owner of the germplasm and has the responsibility of marketing it. Information about the germplasm is distributed by ISURF to companies that may have an interest in **licensing** it for commercial use. If some company indicates an interest, the germplasm is licensed and distributed to them. A **royalty** may or may not be charged by ISURF depending on the germplasm involved.

Some public institutions have an internal committee that reviews the merits of the germplasm and makes a recommendation as to whether or not it should be released. For marketing the germplasm, some institutions contract with a company for that service, instead of doing it themselves.

The decision-making process in private companies generally involves more persons than described for public institutions. The persons at what is commonly referred to as the advancement meeting include the breeders, the individuals responsible for producing seed for sale, and those persons who are responsible for marketing the cultivar.

Legal protection

The two methods established by the US government for protecting germplasm as intellectual property are a patent and a plant variety certificate. A patent gives the developer the right to decide who can grow the germplasm commercially or use it for breeding. A plant variety certificate provides less protection for the developer because it has a research and farmer exemption, which makes it less desirable for the developer than a patent. There are various opinions about whether the research exemption prevents someone from using the germplasm for breeding purposes and has never been tested by the courts. Although the research exemption is not clearly defined, it is very unwise to use any germplasm for breeding without the written permission of the developer, as discussed in "Sources of Parental Germplasm" in *Plant Breeding Methods* (Fehr & Suza, 2024). The farmer exemption for plant variety protection was defined by US Supreme Court in a case involving sale of soybean varieties by a company that was not the developer. The court ruled that farmer's were limited to producing and selling only the amount of seed required to plant their own farmer. For example, if they needed 200 units to plant their farm, only those units of the 200 they did not use for planting could be sold to some other farmer.

It is time consuming and expensive to apply for either a patent or a plant variety certificate and enforcement of the rights provided by each is done by the developer of the germplasm. For those reasons, some developers choose not to use either one. Instead, they have license agreements with companies who produce or market the seed. Those agreements restrict who can produce and sell seed or use the germplasm for breeding.

Branding

In the United States, it is possible to license a cultivar to multiple companies, each of which can give it their own brand name. This same possibility does not exist in other countries, such as Canada, where a cultivar can only be sold by a single name.

There some breeding companies who make extensive use of branding for marketing their cultivars. Although a cultivar can legally have only one cultivar name in the United States, it can be given an unlimited number of brand names. The one restriction is that the buyer must be notified through labeling that it is a brand by using a term such as "Brand XYZ – Variety not stated".

The reason for the popularity of branding is that each company that sells seed of a cultivar can give it a unique designation. The disadvantage for the buyer is that they do not know if the seed from one company may be identical genetically to that of another company. The buyer who wants to plant several different cultivars could end up buying the same one from different companies under different brand names.

Seed Certification

The seed certification system makes it possible for sellers of seed to use a third party to verify the genetic identity and purity of their product to the buyer. Use of the system is voluntary for any seed developer or marketer. The international organization is referred to as the Association of Official Certifying Agencies (AOSCA) http://aosca.org. In the United States, the certification unit in participating states is commonly

referred to as the Crop Improvement Association. The state certification units differ in the services they provide to the seed industry. To understand the services provided by the Crop Improvement Association in a state, simply enter the state name followed by Crop Improvement Association in a search engine, such as Iowa Crop Improvement Association.

One common responsibility of all certifying agencies is to verify the genetic identity and purity of seed through appropriate field and seed inspections. The three classes of certified seed in most states are Foundation, Registered, and Certified. Breeder seed is used to produce the Foundation class. If seed meets the standards for the Foundation class, a white tag can be sewn on the bag of seed. Foundation seed is used to produce the Registered class that is identified by a purple tag, or the Certified class that is identified by a blue tag. The Registered class also can be used to produce the Certified class in some states.

The standards that must be met for certification and the traits that are inspected vary among crops. To understand these standards for any crop, enter the name of the crop followed by Seed Certification Standards in a search engine.

The advantage of seed certification for the buyer is the assurance of knowing that the identity of the cultivar being purchased has been verified by a third party. Companies that do not use the certification system assume that the consumer trusts them to have an internal system to assure that the cultivar name or brand identified with the seed is correct.

Applied Learning Activity

There are no activities for this chapter.

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Walter R. Fehr is an emeritus Charles F. Curtiss Distinguished Professor of Agriculture and Life Sciences, the highest academic honor at Iowa State University (ISU). He obtained graduate degrees in plant breeding from the University of Minnesota and Iowa State University. From 1967 to 2018, he was a faculty member in the Department of Agronomy at ISU where he taught undergraduate and graduate plant breeding courses and conducted research specializing in soybean breeding and genetics. He served as the major professor for 92 students who obtained MS and PhD degrees and authored 270 articles in refereed scientific journals, three books, and 11 book chapters. As a soybean breeder, he developed more than 200 cultivars and was awarded 28 US patents for development of unique genetic traits related to soybean oil quality.

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Walter P. Suza is the George Washington Carver Endowed Chair and an Adjunct Associate Professor at Iowa State University. His research explores the metabolism and physiology of plant sterols, and he teaches courses on Genetics and Crop Physiology in the Department of Agronomy. In addition to co-developing courses for ISU's Distance Master's in Plant Breeding Program, he served for eight years as the director of Plant Breeding E-Learning in Africa (PBEA), expanding access to open educational resources on crop genetic improvement. In 2024, he received the World Food Prize Top Agri-Food Pioneer Award. Suza has worked in central and southern Africa, including with the World Food Programme in Angola and the United Nations Children's Fund in Zimbabwe, focusing on food security assessment, mapping vulnerable households, drought assessment, and coordinating food aid. He holds a Ph.D. in plant sciences, specializing in molecular physiology, from the University of Nebraska-Lincoln.